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(Recognosce notum, ignotum inspice)

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PRESIDENTIAL ADDRESS

MYCOLOGY AND THE WAR

By G. SMITH, M.Sc., F.R.I.C.

It is my privilege to present this address under far happier circumstances than have obtained at several previous Annual Meetings. Although there is still fighting and unrest in many parts of the world, the major war is over and we can now look forward to a gradual improvement in general conditions and, I hope, to an era of steady progress. I have chosen as my subject 'Mycology and the War' because I think it will be useful at this stage to review the past six years from two aspects, the effect of the war on the study of mycology, and the contribution which mycology has been able to make to the war effort.

Although I may claim to be interested in all aspects of mycology, my knowledge is very limited, except perhaps in one specialized field, and I cannot therefore hope to present a picture complete in detail. If, then, I do less than justice to the efforts of some members of the mycological fraternity, it is entirely because I am unable to speak with authority about some of the important war-time activities, and not with any desire to belittle work done in other fields.

When war broke out in September 1939, the prospect for this Society looked decidedly bleak. It was soon evident that this was to be a war of physicists, just as the first World War was a conflict between chemists, and mycologists either simply did not come into the ken of Government officials or were regarded as rare species of the category usually labelled 'suspicious'. The first effect of the war was the cancellation of the programme of autumn forays, and it certainly seemed that any idea of serious field work would have to be abandoned until after the cessation of hostilities. During the first two years there were no official forays and in 1941 there was only a single-day foray at Epping. This was described at the time as a 'token foray', being held, in spite of difficulties, in order to keep alive, though only just alive, one important side of the Society's activities. In the following autumn the two-day foray at the Royal Holloway College was a little more ambitious but still compared very unfavourably with pre-war arrangements. But then came a change, the primary impulse for which was food-rationing. The British public, aided and abetted by a number of more knowledgeable refugees, slowly woke up to the fact that the cultivated mushroom, displayed in the shops at fantastic prices, did not constitute the only means of lending attractiveness to otherwise monotonous dishes, but that there was a wealth of equally attractive food to be had for the picking. Displays of edible and poisonous fungi at South

Kensington during the seasons of 1941-3, and at the autumn shows of the Royal Horticultural Society from 1942 onwards, attracted greater attention each succeeding year. Lectures by Dr Ramsbottom in the South Kensington Museum, which were somewhat sparsely attended in their first season in 1942, drew audiences of continually increasing size, and Women's Institutes all over the country asked for lectures and demonstrations. At the end of 1942 the Foray Committee was set up, with the result that foray programmes, if not the forays themselves, became more ambitious.

The main interest in all this activity, so far as this Society is concerned, is that many, who first began to notice fungi for their possible edible qualities, soon began to desire further, and less utilitarian, knowledge. For many years there has been a great need for more naturalists interested in fungi. There are many points concerning the larger fungi about which we require far more information than is available at present, and this knowledge can best be acquired by large-scale observation. Now that the public is becoming fungus-conscious I do not think it is too much to hope that some of the converts will retain their interest, improve their knowledge and follow in the steps of the distinguished amateurs who have contributed so much to this branch of mycology. In the meantime, the point I wish to emphasize is that the present widespread interest in the larger fungi is directly attributable to the effects of the war, first in creating conditions in which the desire for edible novelties outweighed the traditional British suspicion of all toadstools and, secondly, in bringing into our midst a sufficient leaven of Europeans accustomed to regard many of the despised toadstools as good and valuable food.

Regarding the effect of the war on another important branch of mycology, phytopathology, I think there is little doubt that the science has benefited in several ways from the national drive for enormously increased production of foodstuffs. It was to be expected that more intensive cropping would result in a serious increase in the incidence of disease but, actually, there have been comparatively few unusual outbreaks. The main reason for this immunity is that a long educational campaign by our Research Stations and Advisory Services bore fruit during a period of national emergency. Control measures, such as proper rotation of crops, seed dressing with standardized organo-mercurial preparations, soil sterilization in glass-houses and the use of fungicidal sprays, have all been widely practised as never before. The farmer has come to appreciate, far more than he did before the war, the services which the phytopathologist can render. Once convinced that the scientist can be of real assistance, the agriculturalist himself becomes more scientifically minded and can, in his turn, greatly assist the professional scientist.

The great body of amateurs—gardeners and allotment-holders—have responded to the appeal for bigger and healthier crops in no uncertain fashion. The Advisory Services have been gratified, but also decidedly embarrassed, by the volume of requests by private growers for advice and assistance, and thousands who had never used a spray before have acquired and studied the literature issued by the Ministry of Agriculture and made a determined effort to control pests and diseases. As far as I can

judge, the majority of amateurs are as yet more insect-conscious than fungus-conscious, but this is, I think, quite natural. Perhaps the most serious lapse has been the reluctance to take prophylactic measures against potato blight, sometimes with disastrous results. Also there has been, during the present season a fairly bad epidemic of brown rot of apples, due to *Sclerotinia fructigena*. However, many gardeners are now enquiring what can be done to safeguard the precious apples and it will need very little to convince those who have already found the value of insecticides that the control of diseases is also well worth while.

It is when we come to review the state of industrial mycology during the war that we see the most dramatic development. The two major factors influencing this progress were the spread of the war to the Far East and the resumption of interest in penicillin. Before discussing these, however, I ought to mention one other way in which mycology has been of benefit during the war, namely the development of large-scale production of food-yeast.

It has long been known that the yeast, *Torulopsis utilis*, can synthesize protein from carbohydrate and inorganic sources of nitrogen. The yeast is palatable, much more so than bakers' yeast, the protein it contains is of high biological value, and it is, as well, an excellent source of the B group of vitamins. During the first World War food-yeast was manufactured for human consumption in Germany and again, during the late war, it was made on a large scale, although the only available source of carbohydrate was wood sugar made by a costly process. In this country it has never been made on a large scale. Plans were made, early in the war, to manufacture fairly large quantities from molasses, but this scheme was abandoned because of the difficulty of finding shipping for the raw material. However, after successful semi-large scale experiments had been carried out in England, it was eventually decided to start manufacture in the sugar-producing Colonies, with the double object of finding new uses for sugar against the time when production should once again be in excess of demand, and, in the meantime, of correcting a serious degree of undernourishment amongst large sections of the population in the sugar-growing areas. A company called 'Food Yeast Ltd.' was formed by the Ministry of Food, and Jamaica was chosen for the first large scale venture.

The English pilot experiments were carried out by Thaysen at Teddington and a most interesting stage in the development was the production, by artificially induced mutation, of two new varieties of *Torulopsis utilis*. The first of these, variety *major*, is characterized by its production of cells approximately twice the size of those of the parent strain. The second new strain, variety *thermophila*, is of special value for countries with a high prevailing temperature. During the large scale fermentation much heat is evolved and, unless cooling water of sufficiently low temperature is available, both *T. utilis* and var. *major* are inhibited, their optimum temperature being about 30° C. The new variety *thermophila* grows well at 39° C., permitting the use of cooling water with a temperature as high as 30° C.

The war in the Far East, at an early stage, brought realization to many

of the appalling damage which fungi are capable of doing when they find their ideal environment. Before the war every mycologist knew that the main essential for rapid growth of fungi, and especially of moulds, is abundant moisture, and it was no surprise when information reached this country in 1942 that the destruction of military equipment was on an alarming scale. It has been estimated that, in the early stages of the campaign, less than 50 % of all equipment arrived in usable condition, and some commanders have stated that they considered themselves fortunate if 15 % reached them undamaged. Not all of the damage, of course, was due to the ravages of moulds. Flimsy and unsuitable packing accounted for a large share of the wastage, especially in the beginning, but, as time went on, a larger and larger proportion could be attributed to the activities of moulds and the physico-chemical effects of very high atmospheric humidity.

For many years before the war, some of our greatest industries which manufacture goods for export had been alive to the necessity for protecting materials against mould attack, and valuable work had been done, particularly by the British Cotton Industry Research Association, in defining conditions necessary for growth of moulds and in finding suitable anti-septics. But storage of bales of cotton goods in Indian go-downs in peace time, severe test though it is, is not half so bad as storing military equipment in improvised shelters in tropical forest, where the temperature is very near to the optimum for growth of moulds and the relative humidity is over 90 %, and even close to 100 %, for considerable periods. It is not surprising, therefore, that methods of proofing which were good enough during the era of normal trade should require drastic modification to fulfil war-time requirements.

In the spring of 1943 the Ministry of Supply somewhat belatedly realized that, if our armies were to be suitably equipped for the coming offensive, the mould problem would require some attention. The London School of Hygiene and Tropical Medicine was asked to co-operate and experiments were started in May 1943, although it was nearly another year before some branches of the Services began to realize that there was a problem. After that the volume of work increased to such an extent that it had to be shared between several different establishments. Work along the same lines was also started, almost simultaneously, in Australia and America, and, in order to prevent undue overlapping, there has been, from the beginning, free interchange of information between all three countries. Since I have been directly concerned with this work from the beginning, and as it has had little publicity up to the present, I trust that it will be of interest if I describe it in some detail.

A small chamber was fitted up, in which equipment could be exposed to atmospheric conditions similar to those of a tropical jungle at its worst. The day temperature was fixed at 95° F. at first, but was later lowered to 85° F., and the relative humidity maintained at 98 to 100 %. At night the temperature was allowed to fall, resulting in supersaturation of the air and then condensation of a film of moisture on everything inside the chamber. In the beginning, equipment put in the chamber for test was inoculated with a number of species of moulds, but very soon there was sufficient

mould on many of the specimens, and also on the shelves and the linoleum covered floor, to provide a natural reservoir of infection. Conditions in the chamber were, in some ways, more severe than those of most areas in the war zone, but, on the other hand, there were lacking the typical vegetation and the teeming insect population of tropical jungle.

Besides the testing of complete items of equipment and large samples of materials, the work involved testing hundreds of small samples of an enormous variety of products, and, in addition, the evaluation of many volatile antiseptics and fungicides. For these purposes miniature forcing houses were used, in the shape of vacuum desiccators containing water to maintain a saturated atmosphere. These were incubated in a constant temperature room at 30° C. (85° F.) and materials for test were always inoculated. In both the large tropic chamber and the small glass vessels growth of moulds on susceptible materials was extremely rapid and, after a number of trials with different incubation periods, it was decided to regard as satisfactory any equipment which would remain substantially free from mould for four weeks under these conditions.

For inoculation of specimens it was not easy to make the ideal selection of suitable species but, eventually, six species of moulds were used, and these have been widely distributed to other institutions assisting with the work. The species were *Aspergillus niger* and *A. amstelodami*, both found frequently on mouldy equipment in the tropics and nearly omnivorous; *Penicillium brevi-compactum*, almost as widely occurring; *Paecilomyces varioti*, whose special predilection seems to be bakelite; and two cellulose destroyers, *Chaetomium globosum* and *Stachybotrys atra*. There have been a few surprises in the flora which eventually appeared on some materials, a flora sometimes entirely different from the inoculum. In the early days *Paecilomyces* was not used as a deliberate infection, but it appeared so persistently on samples of sheet bakelite that it was eventually added to the list of test organisms. On one or two occasions *Memnoniella echinata*, described by Bisby, just about the same time, as a 'suspiciously rare fungus', appeared in pure culture, completely covering large sheets of cellulosic material. Several times vigorous growths of *Trichoderma viride* have occurred, particularly on jute fabrics. In general, however, the most common infections recovered from incubated specimens other than fabrics have been the two species of *Aspergillus* and, somewhat less frequently, of *Penicillium*.

I do not intend to catalogue all the various materials which have been tested and for which means of protection have had to be found. They include almost every item of dress, bedding, shelter, weapons and means of communication. There is one class of equipment, however, which has presented a particularly difficult problem, a problem which is not yet completely solved. Optical instruments of all kinds—binoculars, telescopes, microscopes, cameras, range-finders and gunsights—rapidly become unserviceable in the tropics owing to growth of mould on lenses and prisms, and, since there is as yet no way of incorporating an antiseptic in optical glass, they cannot be protected in the same way as most other goods.

The problem is a very old one but, although some attempts were made to

solve it before the war, these efforts were on a small scale and somewhat spasmodic. During the war hundreds of thousands of instruments were in use, with the result that the damage was on such a scale as to make almost impossible demands on the available repair shops, and it became absolutely necessary to make every effort to stop the trouble.

In probably the majority of cases of serious damage, the mould occurring on glass surfaces originates on particles of dust, or on mites which find their way into the instruments. In the days when speed of production was all-important, many binocular bodies were imperfect, threads on focusing screws were often a poor fit and sealing compounds had not always the right physical characteristics. Under these conditions, dust, often of organic origin and carrying spores, readily entered, providing quite sufficient nutriment for appreciable growth of the moulds. However, even with perfect castings, satisfactory screw threads and the best lutings, it was found that mites often gained entry. These creatures are well known to all who keep collections of cultures, as they are very strongly attracted to moulds as a source of food, but, in optical instruments, the roles of predator and prey are often reversed. Mites often enter instruments when no mould is already present and not infrequently die there from lack of food. They carry spores on their hairy bodies, with the result that, after death, they provide much suitable food for the moulds.

Apart, however, from the occurrence of damage which can be accounted for in this way, there have been many cases of serious mould growth in optical instruments when, so far as could be ascertained, there was no foreign matter at all on the glass to serve as food for the fungus. We still do not know how mould can grow under these conditions. Spores will germinate in presence of water alone, but it is extremely doubtful whether the amount of mycelium found in typical cases could result from the material stored in a few spores. If it could not, it is difficult to see whence the mould derives the carbon and nitrogen necessary for growth.

During the war, of course, there was no opportunity for experimental work to solve what is a fascinating but nevertheless academic problem, the urgent requirement being to find some means of keeping glass surfaces free from contamination. Early attempts to keep down the relative humidity inside instruments, by paying careful attention to sealing and by filling with dry air or inert gas at the factory, met with little success. Any optical device with a focusing adjustment inevitably breathes to a certain extent, resulting in the gradual replacement of the dry air by the very humid air of the jungle. The next idea to be tried out was to maintain a fungicidal or fungistatic atmosphere, by means of small quantities of volatile antiseptic suitably placed within the instrument. To contain the antiseptic, very neat little metal capsules were used, made to screw in from the outside so as to permit of ready replacement. They were fitted with diaphragms of sintered glass, in order to retain solid particles whilst allowing free passage of the vapour. The only trouble was to find the perfect antiseptic. Up to the present two substances, metacresol acetate (known as 'cresatin') and thymol, have shown promising results, but cresatin, unless the dose is carefully controlled, tends to corrode metals and both substances soften

canada balsam, so that neither can be regarded as completely satisfactory. Another line of attack has been to use much the same type of metal capsules, but containing 'tell-tale' silica gel. Experiments in this country have confirmed the calculations of the physicists and shown that about half-a-gram of silica gel in each limb of a binocular will keep the internal atmosphere dry for two to three months, even when sealing of the joints is far from perfect. It is still not known, however, whether this method of protection will be adequate if instruments are subjected to fairly rough use under service conditions, and the final solution of the problem was by no means reached when the end of the war came. Research work is, of course, still proceeding, because troops are stationed in the Far East even in normal times, and, for some time to come, will be there in considerable strength.

All this work on tropical proofing needed, at the beginning, some knowledge of taxonomy of moulds and of physiology of fungi, but, when once organized, could be carried out by personnel of comparatively little experience. What will probably prove more important, in the long run, than the contribution which mycology was able to make to the war effort, is the effect of this work on the status of mycology. Interest in mould fungi has spread, not only amongst Service personnel, but amongst manufacturers of all kinds of products. Many manufacturers have, during the war, wished to carry out tests on their own products, tests of suitability to withstand tropical conditions. They have asked for instruction on the methods of carrying out the tests, on detection of mould growth on various materials and, not infrequently, have desired to be able to maintain their own cultures and make their own culture media. A number have asked where they can find literature to supply more information about fungi and have, in fact, become definitely fungus-conscious. As I have already said, a few of our industries were fully alive to the mould problem before the war, but now the interest has spread to manufacturers of such goods as wireless sets, insulated wires and cables, heavy electrical equipment, optical instruments, plastics, and paints and varnishes. The fact that some of these have encountered difficulties in their testing laboratories has made them realize that fungi are living organisms, showing all the vagaries associated with other forms of life, and this has stimulated their interest.

The other field of mycology in which great developments have occurred during the war is the study of the antibiotic activities of fungi. In 1940 appeared the first of a series of papers on penicillin, from the Oxford school under Prof. Florey, showing that this extraordinary substance is active against a number of bacteria *in vivo* as well as *in vitro*, that it is non-toxic and that it does not inactivate leucocytes. Concentrates were prepared and used with a gratifying measure of success in clinical trials, but the low and variable yields of the drug precluded any wider applications at the time. However, wide interest was aroused, both in this country and in America, large-scale research was initiated, and eventually methods of production were so much improved that sufficient penicillin was available just when it was most needed, in the first instance for battle casualties, later for treatment of a limited number of civilian cases.

The predominant part played by the U.S.A. in this development was not due entirely to large resources of money and man-power, but was undoubtedly owing in large measure to an early realization that the initiation of a large-scale mould fermentation is something more than a problem in chemical engineering. From the beginning mycologists were co-partners in the scheme, and to their efforts are due a number of the discoveries which have rendered possible the production of penicillin in adequate quantity and at an economic price. The main obstacles which had to be overcome were the low yield and the difficulty of carrying out the fermentation, on a sufficiently large scale, in shallow layers of liquid medium. The question of the poor yield was tackled by the obvious method of intensive cultural studies and considerable success was achieved thus. At the same time it was thought that it would be most extraordinary if only one strain of a not uncommon species had the ability to produce penicillin. Accordingly, an immense collection of moulds, from all kinds of substrata and from all parts of the world, was assembled and from this miscellaneous collection a large number of strains belonging to the *Penicillium chrysogenum-notatum* series were picked out for test. It was soon found that most strains showed some degree of antibacterial activity and a few of them were very much better than Fleming's original strain. Further, it was not long before a strain was found which would give good yields of the drug when grown in submerged culture. This made it possible to work with large volumes of culture fluid in deep tanks, instead of in numerous shallow vessels of small capacity.

On this side of the Atlantic it took longer to realize that *P. notatum* is a living organism. Several firms went into production without any idea that the services of a mycologist might, to say the least, be useful. It is little wonder that difficulties of all kinds occurred, or that very considerable amounts of penicillin were being produced in America at a time when production in England, its original home, was negligible. At the present time things are much better in this respect, there being quite a number of young chemists and botanists, who have had some training in mycology, now employed on penicillin production.

The importance of penicillin as a means of saving lives during the war can hardly be exaggerated. Its significance from the point of view of the effect of the war on mycology is less obvious but none the less real. It is not only a question of the employment of mycologists in production, and the demand is now greater than the supply, but the effect on the public imagination. Penicillin has become a popular topic of conversation and its production by a common green mould has at least made the average man aware of the existence of these lowly organisms.

Valuable as it is, penicillin is by no means the cure-all it is often imagined to be. Many pathogenic organisms are quite insensitive and it is not surprising, therefore, that intensive search has been made, in recent years, for other products of mould metabolism capable of curing diseases which penicillin does not touch. Several thousand species and strains have been tested but, so far, nothing so outstanding as penicillin has been found, most of the products being of lower antibacterial potency and, at the same time,

more toxic. However, some of them are potentially useful and, meanwhile, the search goes on.

It remains for me now to consider very briefly some ways in which the general interest in fungi, recently stimulated by the conditions of the war years, can be maintained and strengthened.

So far as workers in phytopathology and industrial mycology are concerned, the chief needs are for taxonomists, taxonomic literature and better opportunities for students wishing to take up mycology. I do not need to stress these points, because the report issued and widely circulated last year, on 'The Need for Encouraging the Study of Systematic Mycology in England and Wales', has dealt with the question of taxonomy, and a committee is at present studying the teaching of mycology and will make its report in the near future. As regards the attitude of the public to these subjects, I think more might be done, by means of popular articles and the radio, to keep interest alive. Probably, however, the best way to encourage this developing mycological sense is to do everything possible to stimulate the study of the larger fungi.

One of the chief requirements is for a hand-book in English, written in simple language and with a sufficiency of coloured illustrations. Many beginners are discouraged by the difficulty of trying to identify their finds from descriptions alone, descriptions often bristling with technical terms. Nothing gives more confidence and inclination to persevere, than the positive identification of a few species, and, for the tyro, there is no way so sure as the matching of a fungus with its picture. The writing of such a book depends, to a large extent, on individual inspiration, but the Society could do much to assist in publication when the material is available and might even encourage inspiration.

The other main requirement is for a great extension of field work, and here the Society can take the initiative. During recent years the numbers of forays have increased but the hunting has been, to a large extent haphazard, and I think the time has now come for some more definite work to be attempted. There are many things we should like to know concerning frequency of occurrence, range of variability, conditions which determine fructification, and the reasons for years of abundance and years of scarcity of various species. I venture to suggest that the best way, if not the only way, of initiating work on any problem of this kind is to enlist the aid of young people. The Foray Committee has endeavoured to interest various Natural History societies, but the most that can be expected from any such organization, in any one season, is a single outing devoted to the fungi. What is needed to begin with is the study of a number of comparatively small areas, week by week throughout the season. So far as I know, the only place where this has been attempted at all is at Haslemere, but there the demands of other departments of the Museum have limited the amount of time which could be devoted to fungi. From my own experience I have found that many schoolboys quickly develop a real enthusiasm for toadstools and I am convinced that a little preliminary propaganda in schools would lead to the formation of groups of young students ready to carry out regular surveys of their own neighbourhoods. At first they would, of course,

require some assistance from more experienced fungus-hunters, but that should not be an insuperable difficulty. Apart from the immediate benefits which would result, I think there is little doubt that a real interest in mycology in its wider sense would be aroused in many of the students and that we could hope for more mycologists, and in particular more systematists in embryo, from the next generation of university graduates.

If then we review in a general way the last six years, we see that, not only has mycology been of great service during the war, but appreciation of the importance of fungi has become widespread. It is the responsibility of the British Mycological Society to foster this appreciation and it has been presented with an opportunity such as it has never had before. Next year the Society celebrates its jubilee and I would urge that all who have its interest at heart should honour the occasion by putting forth that extra effort which will ensure mycology attaining its rightful place amongst the sciences.

NOTES ON SOME BRITISH FUNGI ASCRIBED TO *PHOMA* AND RELATED GENERA

By R. W. G. DENNIS, *Royal Botanic Gardens, Kew*

(With Plates I-III and 3 Text-figures)

Among minor diseases of the potato which have received little attention from mycologists are those associated with species of *Phoma*. Three of the latter have been credited with causing disease, namely, *P. solanicola* Prill. & Del., *P. tuberosa* Melhus, Rosenbaum & Schultz, and *P. foveata* Foister. *P. solanicola* was originally described as the cause of a stem rot of potatoes in France by Prillieux and Delacroix (1890), who did not prove its pathogenicity, while Köhler (1928), who rediscovered the species under similar circumstances in Germany, found it non-pathogenic to sound potato stems. The other two species were described in connexion with localized tuber rots and have both been shown by their authors to be feebly parasitic on healthy potato tuber tissue (Melhus, Rosenbaum & Schultz, 1916; Alcock & Foister, 1936). In addition, *P. solani* Cke & Harkness, *P. oleracea* Sacc., *P. nebulosa* (Pers.) Mout. and *P. eupyrena* Sacc. have all been recorded on potato stems. According to Wollenweber (1920) the last named was the cause of 'Pustelfaule' of potato tubers imported to Germany.

Owing to this multiplicity of names applied to pycnidial fungi with hyaline one-celled spores associated with the potato plant, plant pathologists have had much difficulty in identifying the causes of these minor *Phoma* rots. In association with a current investigation into the control of potato dry rot (*Fusarium caeruleum*), a re-examination of the *Phoma* rots was suggested to me by Dr C. E. Foister.

Preliminary isolations soon showed that three types of culture, presumably representing three distinct species of *Phoma*, could commonly be obtained from British potato tubers. As none were aggressive parasites it seemed likely that some or all of them would be found to be soil-inhabiting fungi not specialized as parasites of the potato or any other plant. If so, they might be found also on other hosts, living or dead, and then might have been described under different names on the various substrata. Consequently species of *Phoma* were collected during the spring and summer of 1944, mainly from herbaceous plants in the south-east of Scotland, and as many as possible were taken into culture. In order to economize time and media single-spore cultures were made only from the three types of growth directly associated with the potato tubers, from a *Phoma* on potato haulm and from one on dead stems of *Senecio* which appeared to be of particular interest. With other forms a single pycnidium was carefully picked out with a sterile needle, placed in a drop of sterile water until the spores oozed out and multispore isolations were made from this suspension. These have

the advantage that, at least in the first few transfers, they are more likely to show an 'average' type of mycelial growth than are single-spore cultures, which tend to segregate into extreme mycelial and pycnidial lines (see group II).

The forms studied are listed in Table 1 under strain numbers by which they will be referred to subsequently.

DIFFERENTIAL CRITERIA

In taxonomic work with the Sphaeropsidales the criteria mostly employed have necessarily been mainly morphological, such as the structure of the pycnidia, and the size, shape, septation, mode of production and colour in the mass of the spores. Unfortunately, owing to the small range in spore size shown by the majority of species of *Phoma*, these criteria are not sufficient for identification. Much stress has therefore been placed on the host plant or other substratum with the result that over twenty years ago Shear (1923) could point to the existence of over 1700 so-called species of *Phoma* and remark that 'To refer a species to *Phoma* at present is little more than a confession of ignorance of the organism in hand or its relationship'. Even after segregation of as many forms as possible in *Phomopsis*, *Macrophoma* and similar genera, Grove (1935) still lists 163 British 'species' of *Phoma*, all within a spore range of $2-20 \times 0.5-7 \mu$. In addition are twelve 'species' of *Aposphaeria* and 152 of *Phyllosticta* distinguished from *Phoma* solely by the substratum.

It has been demonstrated, especially by Wollenweber and Hochapfel (1936), that many species of *Phoma* have in fact a wide host range and a correspondingly extensive synonymy. Hence, although it is generally recognized that purely vegetative characters, especially the appearance of the mycelial mat in culture, tend to be less stable than the morphology of the fructification, such mycelial and cultural characters must be taken into consideration, to eke out the meagre range of differential criteria afforded by pycnidia and spores. In the following descriptions detailed accounts are given of the macroscopic appearance of Petri-dish cultures on malt agar (2 % malt extract, 2½ % agar) grown in diffuse light at room temperature (c. 16° C.). The appearance and colour of the mat may vary somewhat with the thickness of the agar layer, which in the present work was maintained at approximately 2 mm. when freshly poured. Microscopic characters of the mycelium seem to be highly variable and hard to define, but the presence of peculiar and abundant chlamydospores is of value in distinguishing *P. eupyrena* Sacc. sensu Woll. and *P. alternariacearum*.

Additional criteria may probably be obtained from physiological characters, such as temperature range and optima for growth, ability to grow on a wide range of media or on the same medium at different pH concentrations and the like. Neither time nor facilities were available to me for such work, but even under the fluctuating temperature of the laboratory the strains could be separated into slow and quick-growing groups. It was possible to compare the isolates as regards their capacity for liquefying 10 % gelatine, as recommended by Grimes *et al.* (1932). At least two parallel

Table 1. Sources of Phoma and other strains used in the investigation

Strain no.	Original host	Organ attacked and date of collection	Current name	Locality	Collector
1	<i>Solanum tuberosum</i>	'Majestic' tuber	<i>Phoma foveola</i> Foister	Aberdeen	Miss E. Gray
2	"	"	? <i>P. tuberosa</i> M. R. & S.	Prestonkirk	Writer
3	"	Last year's stem	<i>P. solanicola</i> Prill. & Del.	Corstorphine	"
4	<i>Senecio Jacobaea</i>	"	? <i>P. herbarum</i>	"	"
5	"	"	"	Greenlaw	"
6	<i>Vicia minor</i>	Dead stem	"	Corstorphine	"
7	<i>Sonchus oleraceus</i>	Last year's stem	<i>Diplodina Sonchi</i> Henn.	"	"
8	<i>Linum usitatissimum</i>	Stem rot	<i>Phoma</i> type } <i>A. linicola</i> Naumoff	Turriff	Miss E. Gray
9	"	"	<i>Ascochyta</i> type } & Vassilievski	"	"
10	<i>Lonicera Periclymenum</i>	Living leaf	<i>Phyllosticta Lonicerae</i> Westd.	Cupar, Fife	Writer
11	<i>Lycopersicon esculentum</i>	Stem	<i>Diplodina Lycopersici</i>	Received from Cheshunt	"
12	<i>Hedera helix</i>	Living leaf	<i>Phyllosticta hedericola</i> Dur. & Mont.	Exp. St.	"
13	<i>Fraxinus excelsior</i>	Fallen fruit	? <i>Phoma Aceris-negundinis</i> Arc.	Bridge of Allan	"
14	"	"	Uncertain	Greenlaw	"
15	<i>Centaurea Scabiosa</i>	Last year's stem	? <i>Phoma herbarum</i>	Corstorphine	"
16	<i>Ribes Grossularia</i>	Living leaf	<i>Phyllosticta Grossulariae</i> Sacc.	Greenlaw	"
17	<i>Solanum tuberosum</i>	'Majestic' tuber	<i>Phoma auyreana</i> Sacc.	Achnashellach	Miss E. Gray
18	"	Tuber	"	Prestonkirk	Writer
19	<i>Heracleum Sphondylium</i>	Last year's stem	"	England	A. R. Wilson
20	<i>Urtica dioica</i>	Base of last year's stem	<i>P. complanata</i> Desm.	Corstorphine	Writer
21	<i>Sisymbrium officinale</i>	"	<i>P. acuta</i> Fekl.	"	"
22	Unidentified Umbellifer	"	Uncertain	"	"
23	<i>Lapsana communis</i>	"	Uncertain	Pass of Leny	"
24	<i>Anthriscus sylvestris</i>	"	? <i>Phoma Lampanae</i> Karst	Ardeonaig, L. Tay	"
25	<i>Urtica dioica</i>	"	Possibly <i>P. sanguinolenta</i> Grove	Pass of Leny	"
26	<i>Angelica sylvestris</i>	Last year's stem	? <i>P. Urticae</i> Sch. & Sacc.	Corstorphine	"
27	Cream	"	? <i>P. oleracea</i> Sacc.	Balquhider	"
28	<i>Urtica dioica</i>	"	<i>P. hibernica</i> Grimes <i>et al.</i>	Cork, Eire, June 1944	Prof. M. Grimes
29	<i>Scrophularia nodosa</i>	Last year's stem	<i>P. nebulosa</i> (Pers.) Mont.	Callander	Writer
30	<i>Solidago Virgaurea</i>	"	<i>P. oleracea</i> Sacc.	L. Lubnaig	"
31	<i>Galium aparine</i>	"	? <i>P. herbarum</i> f. <i>Solidaginis</i>	Glen Lednock	"
32	<i>Ribes Grossularia</i>	Bark of living twig	<i>Diplodina Gallii</i> (Niessl) Sacc.	Greenlaw	"
33	Swedish turnip	'Root'	<i>Diplodina Grossulariae</i> Sacc. & Bri. sensu Grove	Greenlaw	"
34	<i>Lycopersicon esculentum</i>	Fruit	<i>Phoma lingam</i> (Fr.) Desm. <i>P. alternariacearum</i> Brooks & Searle	Corstorphine Received from Prof. Brooks	"

tubes were inoculated with each strain and maintained at room temperature. The rate of liquefaction by different isolations of the same fungus was sufficiently constant for this character to be regarded with some confidence as a differential criterion.

Degree of pathogenicity to selected host plants may also afford a useful clue to the relationship of any particular isolate. It has to be remembered, however, that it is not unusual to find non-parasitic or weakly parasitic strains of common fungus parasites and also that pathogenicity may decline with prolonged cultivation on artificial media. Thus Foister's original isolate of *P. foveata*, maintained in the National Collection of type cultures, was found to be no longer pathogenic to potato tubers. Similarly the culture of *P. alternariacearum* received from Prof. Brooks would not attack tomato fruit.

By the use of the above criteria the thirty-four strains have been classified in seventeen groups. Whether or not these represent 'good' species will be a matter of opinion. Thus in group II strains 2, 3, 4 and 5 appear to be identical, strain 6 seems to be a less active parasite, strain 7 had a higher proportion of septate spores in the original collection, strain 9 differs slightly in appearance of the mycelial mat, while strains 8 and 9, though of the same general type, differ from the others in pathogenicity to flax. It is obviously undesirable to attempt to allot valid names to any of the groups until all or nearly all the described species of *Phoma* have been studied in culture. Any name allotted at present will be liable to be invalidated by the discovery that an earlier described species on a different host fell into the same group of strains. The nomenclature adopted is therefore, as far as practicable, that of Grove (1935), which seems likely to be in use in Britain for many years.

INOCULATION EXPERIMENTS

(a) *On potato tubers.* All inoculations were made on tubers of the variety Doon Star, which is known to be particularly susceptible to the 'gangrene' type of *Phoma* rot. They were made by inserting a minute piece of an agar culture under the small flap of skin left by a cut 2-3 mm. long on the surface of the washed tuber. Inoculated tubers were kept under bell jars on the surface of moist peat or sphagnum. During spring and early summer of 1944, tubers of a 1943 crop were inoculated with strains 1-6, 11, 13-15, 17-34, while on 18 September 1944 freshly lifted tubers were inoculated with strains 7-10, 12 and 16. This point is of importance, since it is known that the susceptibility of potato tubers to Dry Rot and Gangrene appears to increase during storage over winter, which must be taken into account in comparing the lesions produced on the two seasons' crops.

There were three types of reaction on potato tubers. In the majority of cases no invasion of the tissues took place, though the flap of skin usually shrivelled. In the second type, exemplified by strains nos. 1, 2, 3, 4, 5, 6, 7 and feebly by 8, 9, 10 and 13 a small, more or less circular, hard, dark rot developed. This usually became sealed off in a few weeks and extended no further in diameter, though the depth might be successively extended to

about 1 cm. The dried-up dead tissue could usually be prised out after about one month, leaving a clean healed cavity behind. This is the button type of rot, which, especially with strain 1, occasionally progressed more deeply, with formation of cavities in the dead tissue, as in typical gangrene. Pycnidia developed on the lesions formed by strains 1, 2, 3, 4, 5 and 7. The third type of reaction, shown only by *Diplodina Lycopersici*, was a rather rapid but shallow subepidermal rot which rapidly spread over the surface of the tuber. Mature pycnidia were produced on the diseased tissue and yielded spores from which the fungus was easily reisolated.

(b) *On swede roots.* Mature purple-topped swede roots were obtained from a clamp in the spring of 1944 and inoculated in the same way as the potato tubers. The same three types of reaction were shown, namely: no invasion; localized circular lesions by strains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 13; and extensive rot by *Phoma lingam*, strain 33, only. Pycnidia were produced on swede roots only by the latter. A few strains had to be tested on roots fresh from the field in September 1944 and, as with potato tubers, the lesions produced were smaller than those on the overwintered roots. Thus strain 2, which formed lesions 6–12 mm. across on roots inoculated 28 March 1944, produced them only 4 mm. across on roots inoculated 7 September 1944. In neither instance did the lesions increase in size after the first week. Even lesions only 4 mm. across, however, are quite distinct from the negative reaction given by most strains.

(c) *On tomato stems.* Young tomato plants about 12–18 in. high were obtained in individual pots from a local nursery and inoculated in the stem about 2 in. above soil level in the same way as potatoes and swedes. At the point of inoculation the stem was then wrapped round with a strip of oiled paper which was removed after three or four days. Here again most strains induced no lesions. Others formed a small rather dry canker. If this developed far enough to girdle the stem, the plant wilted and died (Pl. II, fig. 11). If not, the canker dried up and the plant grew normally, apart from the production of adventitious root rudiments above the lesion. Girdling and death took place with some inoculations of strains 1, 2, 3, 4, 5, 7, 8 and 11, cankers without girdling with strains 6 and 13. No sharp distinction can be drawn, however, between these two groups, as of a number of inoculations made at the same time from the same culture one might result in girdling and the others not, even in strain 11. Pycnidia developed on the lesions caused by strains 1, 2, 3, 4, 5, 7, 11 and 13. Other plants of similar size were sprayed with spore suspensions of strains 1, 2, 3, 4, 6 and 11. The results of this experiment were less clear cut and are described under groups I, II and III.

(d) *On tomato fruit.* Picked fruit stored in a single layer on the floor of a large closed tin were inoculated by cuts in the usual way when fully grown and just turning yellow. A clear-cut distinction can be drawn between strains which rotted the fruit rapidly and those which were completely non-parasitic. Positive results were obtained with strains 1, 2, 3, 4, 6, 7, 8, 9, 10, 11 and 13. Strain 5 was not tested (Pl. III, fig. 3).

(e) *On apple fruit.* This host was chosen to facilitate comparison with the species described by Wollenweber and Hochapfel (1936). Unfor-

tunately the only variety of which fruit were available to the writer in sufficient quantity free from other diseases, especially scab, was a little-known one, called locally Ben's Red. This may have been somewhat resistant to *Phoma* rots (as it appears to be to scab), as in many cases the rot did not proceed beyond a small lesion 3 or 4 mm. in diameter round the point of inoculation. Other inoculations, often with the same strains as those which remained thus circumscribed, produced a rather slow brown rot, over the central portion of which developed a black crust of hyphae but

Table 2. Results of inoculation experiments with *Phoma* strains

Strain	Potato tuber	Swede root	Tomato stem	Tomato fruit	Apple fruit
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	?
5	+	N.T.	+	+	N.T.
6	+	+	+	+	?
7	+	+	+	+	+
8	+	+	+	+	?
9	+	+	N.T.	+	?
10	+	+	? +	+	+
11	++	+	+	+	?
12	-	-	-	-	-
13	+	+	+	+	?
14	-	-	-	-	-
15	-	-	-	-	-
16	-	N.T.	N.T.	-	-
17	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	-	-	-	-	-
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	-	-	-	-	-
26	-	-	-	-	-
27	-	-	-	-	-
28	-	-	-	-	-
29	-	-	-	-	-
30	-	-	-	-	-
31	-	-	-	-	?
32	-	-	-	-	-
33	-	++	-	-	-
34	-	-	-	-	-

no mature pycnidia. In the table above the former type of reaction is indicated by a ?, the latter, even if only from a few inoculations, by a +. Foister (unpublished observations) has previously shown *P. foveata* to be able to rot apple fruit.

The results of all these inoculation experiments are collected in Table 2. The three types of reaction on tubers and swedes are indicated by -, + and ++, those on tomato stems and fruit by - and +. N.T. signifies not tested. Unless otherwise stated, potato tuber observations are based on ten inoculations or more (see Table 4), those on the swede on five inoculations on the same root, those with tomato stems on two plants, and with

tomato and apple fruit on two inoculations on opposite sides of each of two fruit.

DESCRIPTIONS OF THE INDIVIDUAL STRAINS

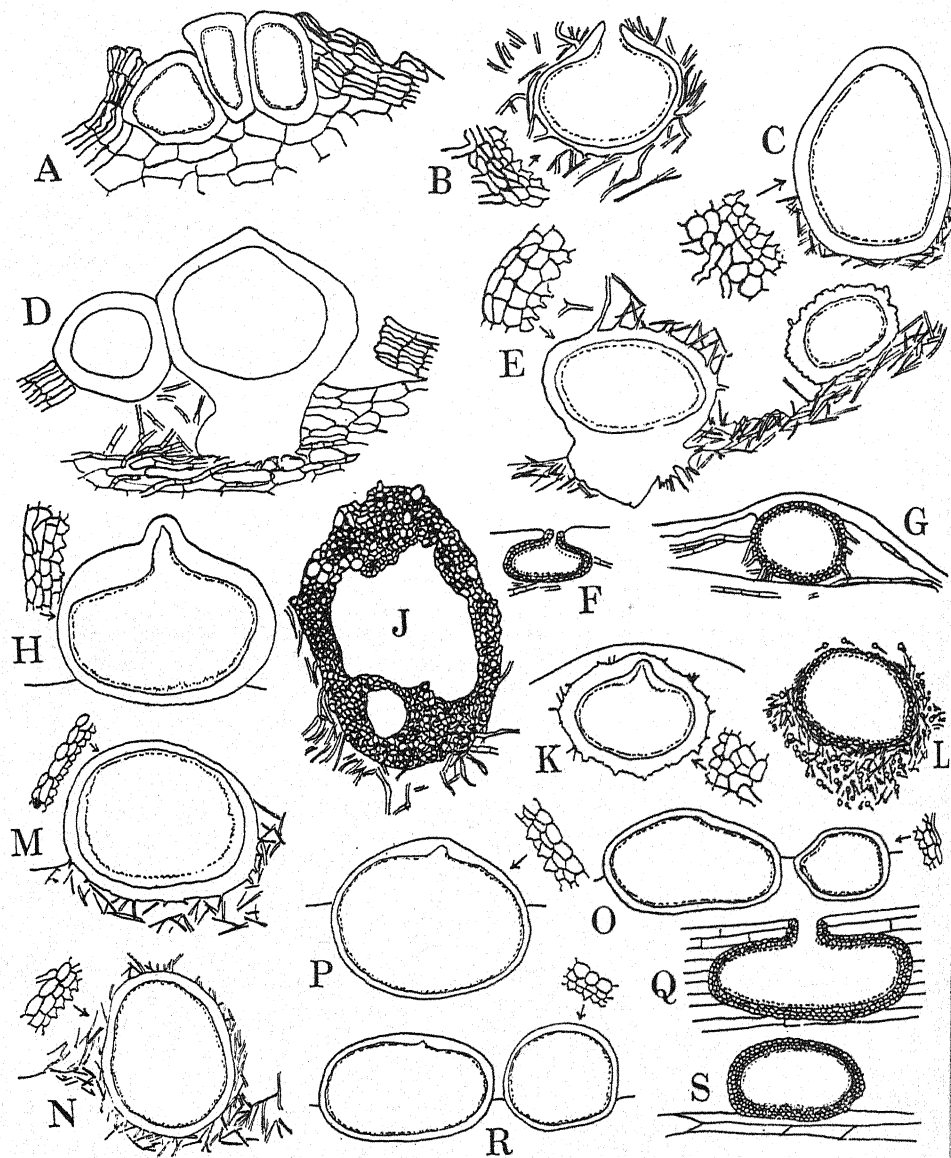
Group I. Phoma foveata Foister. For the first three days of growth there is a short, mealy, white aerial mycelium without zoning. By the fourth day a light yellow-brown tint appears at the centre of the culture and by the fifth day dark, submerged hyphae are present in irregular brown patches forming a zone of inner diameter 5–6 mm. As the culture grows the colour becomes intensified until the entire mat appears teak colour, with indistinct zones of greyish white aerial hyphae (Pl. I, fig. 1). Over the central area the colour of the aerial mycelium itself is yellow-brown, but the colour of the whole culture is dominated by that of the dark submerged hyphae showing through the sparse mat. When viewed from below the distinct, though irregular and discontinuous concentric belts of dark olive-brown hyphae are characteristic of all isolations referred to this species (Pl. I, fig. 2). Mature pycnidia are not found on agar plates, but scattered sclerotial bodies interpreted as immature pycnidia occur. Mature pycnidia occasionally develop on old malt-agar slants (Text-fig. 1 B). Brush streak cultures with a spore suspension on agar plates yield the same type of growth. An irregular, broken line of immature pycnidia develops along the streak, but there is no distinct clear belt on each side of this as in group II. The remainder of the culture is occupied by alternating zones of light and dark submerged hyphae like those on plates inoculated at the centre in the usual way.

On sterilized potato plugs the fungus forms a dense, short, pinkish brown mycelial mat in which are embedded innumerable clusters of black pycnidia (Text-fig. 1 A). At the upper dry end of the plug the growth is looser, more erect and grey-brown with white hyphal tips. Where the edge of the plug impinges on the glass of the tube a dark brown, almost chocolate-coloured band is visible with a fringe of similar coloured hyphae growing over the glass surface.

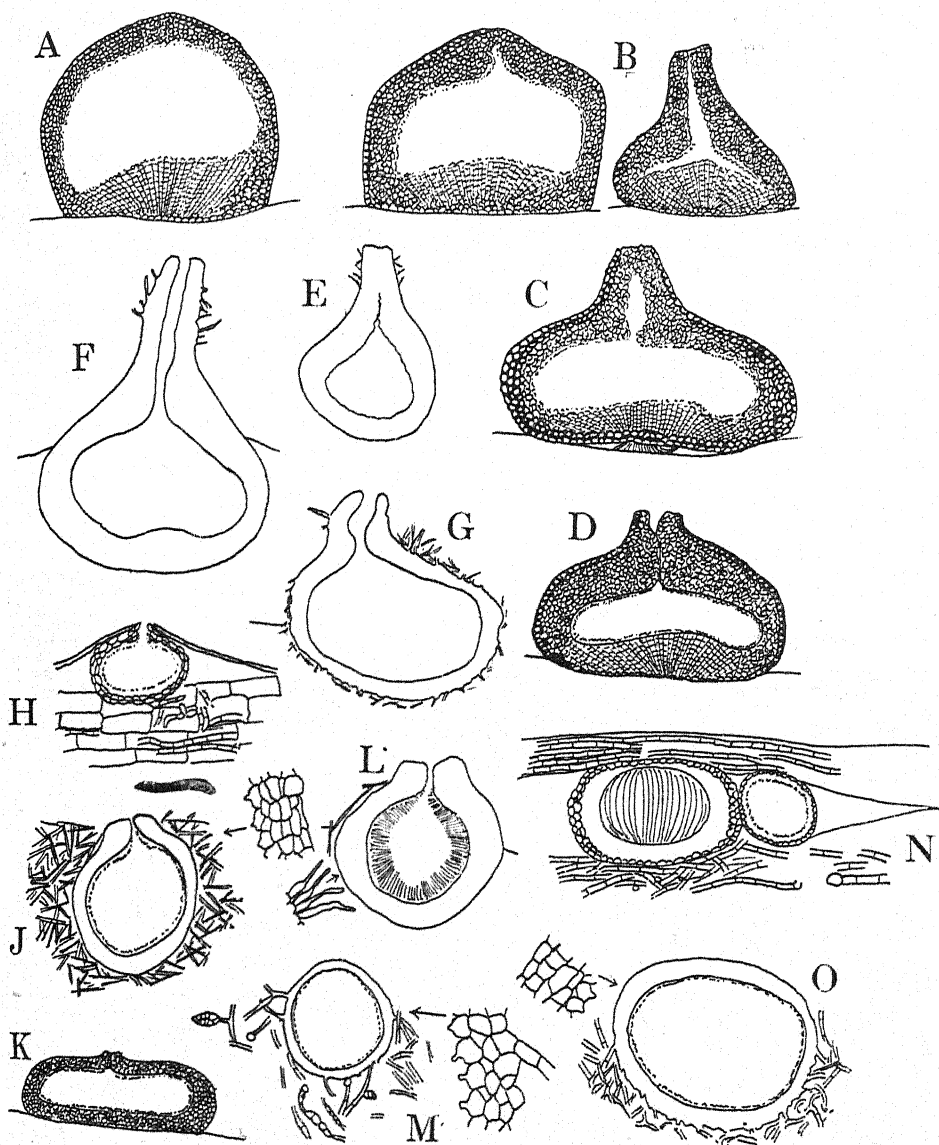
Strain 1 liquefied gelatine to the extent of 12.5 mm. in fifteen days and 21 mm. in thirty days. Foister's original isolation of *P. foveata* received from the National Collection of Type Cultures differed from the above in having little or no aerial growth and in producing pycnidia very freely on malt agar slants.

Strain 1 was parasitic to Doon Star tubers, on which it caused button type or small gangrene lesions of mean diameter 17.4 mm. On these pycnidia were produced in abundance, singly or fused into irregular groups arranged in more or less distinct concentric circles. They were black, immersed or almost superficial and exuded a pink exudate which dried carrot colour. According to the diagnosis, *P. foveata* has spores 'yellow-cream' in the mass (Foister, 1940). The colour of the exudate in the original isolation now corresponds, however, with that of strain 1 which is accepted as *P. foveata* by Foister.

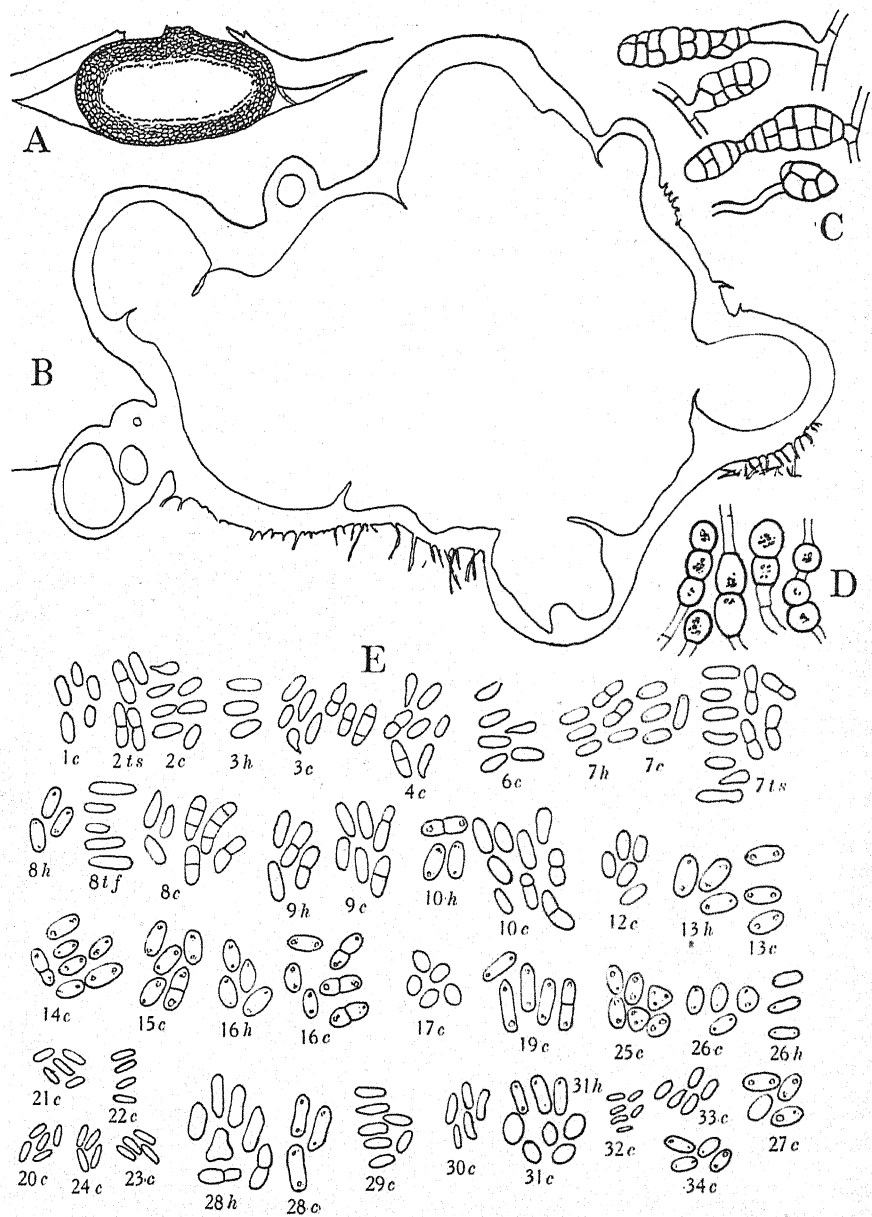
The spores are colourless, cylindrical with rounded ends and non-



Text-fig. 1. Vertical sections of pycnidia, from camera lucida tracings to the same scale, all $\times c. 70$. Details of pycnidial wall, $\times c. 500$. Unless otherwise specified, all cultures referred to are on malt agar. A. Strain 1 on potato tuber. B. Strain 1 in tube culture 111 days old. C. Strain 3 in 6 weeks old culture. D. Strain 2 on potato tuber. E. Strain 4 in 65 days old culture. F. Strain 4 on *Senecio* stem. G. Strain 3 on old potato stem. H. Strain 12 in 5 weeks old culture. J. Strain 11, immature pycnidium in 55 days old culture. K. Strain 19 in 55 days old culture. L. Strain 18 in culture, pycnidium embedded in mycelium full of chlamydospores. M. Strain 13 in 40 days old culture. N. Strain 14 in 28 days old culture. O. Strain 27 in 37 days old culture. P. Strain 25 in culture 55 days old. Q. Strain 25 on *Urtica dioica* stem. R. Strain 26 in culture 26 days old. S. Strain 26 on *Angelica* stem.



Text-fig. 2. Explanation as Text-fig. 1. A. Strain 21 on *Sisymbrium* stem. B. Two pycnidia of strain 24 on *Anthriscus* stem. C. Strain 22 on the host. D. Strain 20 on *Urtica dioica* stem. E. Strain 22 in culture 53 days old. F. Strain 24 in culture 57 days old. G. Strain 21 in culture 55 days old. H. Strain 30 on *Solidago* stem. J. Strain 30 pycnidium embedded in mycelium on potato plug culture 28 days old. K. Strain 19 on *Heracleum* stem. L. Strain 32 in culture. M. Strain 34 in culture 11 weeks old. N. Strain 28 on *Urtica* stem, pycnidium in contact with perithecium of *Didymella* sp. O. Strain 28 in 28 days old culture on potato plug.



Text-fig. 3. A. Pycnidium of strain 29 on *Scrophularia* stem, $\times 70$. B. Multilocular fructification of strain 29 in culture 28 days old, $\times 70$. C. Chlamydospores of strain 34, $\times 500$. D. Chlamydospores of strain 17, $\times 500$. E. Pycnosporos ($\times 500$) of the strains indicated by number. *h*=from the host; *c*=from artificial culture; *ts*=from tomato stem; *tf*=from tomato fruit.

septate (Text-fig. 3 E 1). The dimensions are somewhat larger than those cited by Foister, viz.:

From pycnidium on 3 months old agar slant	7.5 × 2.9 (5.6–9.3 × 2.2–3.6 and up to 4.8) μ
On Doon Star tuber	6.6 × 2.9 (4.8–8.2 × 2.8–3.4) μ
On tomato stem	7.5 × 3.3 (5.0–11.5 × 2.8–3.9) μ
Foister's diagnosis (from tuber)	5.7 × 1.7 (3.2–7.7 × 1.1–2.1) μ

The mycelium in culture varies from hyaline to light brown, composed of elongated cells typically crowded with oil globules. In old submerged hyphae individual cells commonly round off to form long-elliptical chlamydospores which may germinate to give rise to one or more narrower hyphae within the original hyphal wall.

When inoculated to tomato stems strain 1 caused girdling and falling over of the plant in nine days, followed by copious production of pycnidia on the lesion. Spore suspension sprayed over two tomato stems on 23 May caused an occasional leaf spot on the lower fading leaves, but no apparent stem lesion. Pycnidia developed on these leaf spots when the leaflets were picked and kept five days in a damp Petri dish. Strain 1 was successfully recovered from these pycnosporos. Both plants, however, wilted and died simultaneously about two months later while control plants were still vigorous and strain 1 was reisolated from the dead stem tissue. Green tomatoes were rotted freely, lesions 12 mm. in diameter being formed in seven days. Inoculation to apple yielded a somewhat slow brown rot on which immature pycnidia began to appear after four weeks. Foister's original isolate of *P. foveata* was no longer pathogenic to potato, tomato fruit or to apples, and formed only small cankers on tomato stems.

Isolates identical in appearance with strain 1 were obtained from Majestic tubers from Prestonkirk, Ninetyfold and Doon Star from Dumfries, Craigs Defiance and a seedling from Edinburgh, and were also received from Drs Boyd and Wilson at the Midland Agricultural College.

Group II. In this group are included strains 2–10 inclusive, isolated from potato, ragwort, periwinkle, sowthistle, flax and honeysuckle. These all behaved similarly in culture and gave similar though not identical reactions when inoculated to the differential hosts. All are liable to produce a number of 1-septate spores, but the percentage of these fluctuates considerably between different isolates and even with the same isolate on different substrates and in pycnidia of different ages. Thus no septate spores were found in the exudate in a single-spore culture of strain 4 five weeks old. When it was eight weeks old about 1% of the exuded spores were septate. Hence some of the strains may be classified as *Phoma* or *Phyllosticta*, others as *Diplodina* or *Ascochyta*. All may not ultimately be considered to belong to a single species, but they appear to be closely related and to form a natural group.

Growth in culture begins as a rather loose white aerial mat, which straggles up to a height of 3 mm., and develops rather characteristic tangential white crests round the margin on the second day. By the fifth day the central mat becomes closer, shorter and takes on a grey tinge, sur-

rounded by a dense white belt with an irregular extending margin which is already distinctly crenate. By the sixth day olive-grey tangential streaks appear among the submerged hyphae apparently corresponding in position to the white crests of the second day's growth. The culture darkens rapidly and by the eighth day the central area appears solid black below, covered by a straggling grey aerial mat. By the twelfth day almost the whole surface of the culture is covered with a dense olive-grey mat with looser whitish tips. Between this and the narrow whitish marginal zone is usually a belt where the dark submerged hyphae are visible from above, owing to the sparseness of the aerial mat. By this time the margin is usually very irregular, owing to growth taking place irregularly over short arcs, corresponding to which are forked and branched tongues of blackish submerged mycelium. Vague zoning may be apparent in the submerged growth and black hyphal knots and immature pycnidia appear (Pl. I, figs. 4-6).

On potato plugs there is a dense even coat of dark grey mycelium, looser and paler at the upper end and forming an almost black band where the plug is in contact with the glass. Pycnidia were abundantly produced by strain 2 on this substrate, but on agar plates and slants pycnidial production was somewhat irregular. The original mycelial isolation of strain 2 produced very few pycnidia on malt agar, though hyphal knots were frequent especially along the glass of tube cultures. A line of ripe pycnidia developed, however, all round the margin of a slant which had been removed from its tube and left for eight days in a dry Petri dish. Single spore cultures made from the exudate of this strain on a potato plug segregated into two groups, one showing predominantly mycelial growth with only occasional pycnidia, the other less copious aerial mycelium and abundant pycnidia. This is evidently an instance of the 'Dual Phenomenon' described by Hansen (1938) in *Phoma terrestris* and other imperfect fungi. The difference between the two groups remained constant over a series of transfers, but it is somewhat surprising that of seven single-spore isolations made from a single pycnidium of each of strains 3 and 4 those of the former were all of the mycelial type and those of the latter all pycnidial. The remaining strains of which multispore isolations only were made produced pycnidia somewhat sparsely in culture. In strain 6 pycnidia were produced round the edge of a hole in the culture from which inoculum had been taken.

Brush streak cultures of strain 2 with a spore suspension from potato plugs gave copious pycnidial formation down the line of the streak, which was marked by an irregular belt of dark submerged hyphae bordered on each side by a narrow, barely continuous, clear zone. Beyond this the plate was covered with the normal grey mycelial growth covering blackish submerged hyphae and almost devoid of pycnidia. The clear bands were about 1 mm. wide, about 15-16 mm. apart, and scarcely discernible from above. Otherwise the appearance resembled that described by Köhler (1928) for *P. solanicola*. Strains 2, 3 and 4 grown together on the same plate appeared identical (Pl. I, fig. 4). Strain 10 gave a rather lighter grey aerial mat and less dark submerged mycelium than the others and may possibly be distinct (Pl. III, fig. 6).

Growth rates of the strains of group II varied considerably as did those

of the same isolate at different transfers or even on plates made simultaneously from the same inoculum. Most strains grew fairly quickly for the first three or four days, after which the growth rate fell off with initiation of the crenate margin and localized extension of individual arcs. Growth sometimes ceased altogether before the margin of the plate was reached.

Spores of all strains are cylindrical with rounder ends or slightly narrowed to one end, but somewhat irregular spores are not infrequent. There is only very slight constriction at the septum when it is present. In all cases the spore exudate is pink. The spore dimensions of individual strains grown on different substrates are set out in Table 3, which also includes the spore dimensions of a fungus isolated from basal lesions of flowering *Brassica sinapis* plants from a flax field at Errol, Perthshire. It appeared to belong to group II, but was not further studied. Occasional 2-septate spores measuring $11-14.6 \times 3.9-4.8 \mu$ were seen in strains 5, 8 and 9 and the Charlock fungus, and a solitary 3-septate spore measuring $14.4 \times 3.9 \mu$ in strain 8.

The behaviour of the group II strains when inoculated to Doon Star tubers has been summarized in Table 2, but is set out in more detail in Table 4, along with the data on rate of liquefaction of gelatine.

All strains produced the same type of localized lesion on swede roots, seldom exceeding 1 cm. in diameter. All rapidly rotted green tomato fruit and all produced cankers on tomato stems, but whereas at least some inoculations with strains 2, 3, 4, 5, 7 and 8 led to lesions which girdled the stem and killed the plants, none of strains 6 and 10 did so. Strain 9 was not tested on tomato stems. Pycnidia were produced on the cankers of strains 2, 3, 4, 5 and 7.

With a view to investigating the relationship of the group II strains to group III (*Diplodina Lycopersici*) a number of young tomato plants were sprayed with spore suspensions of strains 2, 3, 4, 6 and 11. The results may be summarized as follows:

Strain 11: Grey foliar lesions 5 mm. across with concentric zones and ripe pycnidia in five days. Stem lesions apparent and lower leaves wilted in 17 days, plants eventually killed.

Strain 2: Grey lesions on leaves and fading cotyledons with ripe pycnidia in 9 days. Three small stem lesions up to 3×1 mm. after eleven days. No further development.

Strain 3: Grey lesions on leaves and cotyledons with ripe pycnidia in 9 days. No stem lesions, but a solitary lesion near base of a petiole in eleven days.

Strain 4: Foliar lesions in six days with pycnidia in nine days from which the strain was recovered in culture. Eight stem lesions up to 10×2 mm. after eleven days, but these developed no further. Four pycnidia were noted on them after twenty-three days.

Strain 6: Small grey lesions up to 2 mm. across on the second and third leaves only, no pycnidia and no stem lesions.

It will be seen that though strains of this group are capable of attacking uninjured leaves of young and vigorous tomato plants they are all much less aggressive than *Diplodina Lycopersici*.

In this connexion it is worth recording that in the case of a tomato plant

Table 3. Spore characters of the strains in Group II (Text-fig. 3 E)

Strain	Substrate	Size of non-septate spores	1-septate spores	Approx. % septate
2	Original isolate	6.3×2.6 ($5.9-8 \times 2.2-3.1$) μ	—	0
	Pycnidial type	7.0×3.5 ($4.8-8.7 \times 2.8-4.5$) μ	—	0
	Mycelial type	7.0×3.6 ($5.0-10.1 \times 3.1-5.0$) μ	—	0
	Tomato stem	8.0×3.6 ($7.10 \times 3.4-2$) μ	10.6×3.5 (9.12×3.4) μ	30
3	Agar 8 weeks old	7.9×3.2 ($6.4-10.1 \times 2.8-3.9$) μ	9.6×3.6 ($8.4-11.0 \times 3.3-4.2$) μ	8
	Potato haulm	8.0×3.4 ($6.7-9.0 \times 2.8-4.0$) μ	—	0
	Tomato stem	7.5×3.4 ($5.0-10.1 \times 2.8-3.9$) μ	10.6×3.8 ($10.1-11.3 \times 3.3-4.5$) μ	3
4	Agar 8 weeks old	7.2×3.5 ($5.0-9.6 \times 3.1-4.2$) μ	9.6×3.5 ($8.5-10.7 \times 3.1-4.2$) μ	1
	Tomato stem	8.4×3.6 ($5.9-10.7 \times 2.8-4.2$) μ	10.3×4.0 ($9.12.5 \times 3.3-4.8$) μ	7
5	Agar 9 weeks old	6.6×3.5 ($4.2-8.7 \times 2.5-3.6$) μ	8.6×3.7 ($7.6-10.4 \times 3.1-3.9$) μ	less than 1:1000
	Agar 7 weeks old	6.3×3.1 ($4.5-8.2 \times 2.8-3.7$) μ	—	0
6	Agar 7 weeks old	6.6×3.2 ($4.7-9.0 \times 2.5-3.9$) μ	—	0
	Sonchus stem	7.4×3.2 ($5.6-9.3 \times 2.8-3.9$) μ	9.7×3.7 ($7.6-11.8 \times 3.3-3.9$) μ	7
	Tomato stem	8.5×3.2 ($6.7-10.2 \times 2.8-3.7$) μ	10.3×3.5 ($9.3-12.4 \times 3.3-3.7$) μ	2
	Flax stem	7.5×3.3 ($4.2-10.1 \times 2.8-3.7$) μ	10.0×3.8 ($8.5-11.8 \times 3.3-4.2$) μ	1
7	Tomato fruit (crushed pycnidia)	6.5×3.1 ($5.6-7.6 \times 2.8-3.6$) μ	—	0
	Agar 9 weeks old	7.9×3.4 ($5.9-10.4 \times 2.2-4.2$) μ	—	0
	Flax stem	Not measured	10.8×3.4 ($9.0-13.8 \times 2.8-3.9$) μ	3
	Tomato fruit (crushed pycnidia)	7.7×3.1 ($5.0-11.0 \times 2.8-3.7$) μ	10.0×3.7 ($8.7-11.0 \times 3.1-4.2$) μ	11
8	Agar 9 weeks old	6.8×3.5 ($5.0-10.1 \times 3.1-4.2$) μ	9.6×4.2 ($7.9-12.7 \times 3.3-4.5$) μ	less than 1
	Flax stem	7.5×3.7 ($6.4-8.5 \times 3.1-4.2$) μ	—	?
	Honeysuckle leaf	6.4×3.0 ($5.6-8.5 \times 2.5-3.6$) μ	$9.5-10.4 \times 3.9-4.2$ μ	?
	Tomato fruit (crushed pycnidium)	7.2×3.0 ($5.0-9.0 \times 2.5-3.7$) μ	—	0
9	Agar 6 weeks	—	9.4×3.4 ($8.4-11.3 \times 3.1-3.7$) μ	?
	fungus	—	—	?

sprayed with *D. Lycopersici* spore suspension on 23 May and killed by a girdling lesion about $1\frac{1}{2}$ in. above soil level, a secondary shoot sprouted from the axil of the second leaf, a short distance below the lesion. By 20 June this shoot was also killed and the entire stem down to soil level was eventually rotted. In a similar plant, girdled and killed after inoculation with the *Senecio* strain 5 on 16 April three such secondary shoots developed and remained healthy and vigorous until the plant was discarded at the end of July. Lesions induced by strains of group II evidently become localized either before or after girdling has taken place, whereas the tomato is unable to localize infection by *D. Lycopersici*.

Lesions were produced on apple fruit by all strains of this group, but whereas those of strains 4, 6, 8 and 9 remained localized and did not exceed 4, 5, 5 and 5 mm. in diameter respectively, those of strains 2, 3, 7 and 10

Table 4

Strain no.	Depth of gelatine liquefied in		Date of inoculation	Doon Star tuber inoculations			
	15 days	30 days		No. of successful inoculations	Total no. made	Mean diameter of lesions mm.	Range in diameter of lesions mm.
	mm.	mm.					
2	$10\frac{1}{2}$	21	10. 2, 27. 3 and 18. 4. 44	30	30*	9.6	6-13
3	$9\frac{1}{2}$	19	27. 3 and 18. 4. 44	14	14*	10.7	8-15
4	$8\frac{1}{2}$	17	29. 3. 44	24	24*	10.0	7-11
5	11	23	16. 5. 44	7	10*	7.6	4-12
6	$10\frac{1}{2}$	21	16. 5. 44	6	10	4.0	2-9
7	14	23	10. 6. 44	9	10*	6.3	3-10
8	13	26	18. 7 and 18. 9. 44	?	22	3.0	2-5
9	—	—	18. 9. 44	?	12	3.0	2-4
10	12	24	18. 7 and 18. 9. 44	?	22	3.0	2-4

It will be observed that the strains appear to form a series of decreasing pathogenicity to potato tubers, until in the case of strains 8-10 it is not always possible to decide whether a small lesion has been formed or not. It must be remembered, however, that these were tested only on freshly dug tubers or on shrivelled specimens kept in store till mid-July. No difference in pathogenicity was shown by the mycelial and pycnidial lines of strain 2, which gave lesions of mean diameter 9.3 and 10.1 mm. respectively. Mature pycnidia were formed on the lesions produced by the strains indicated by an asterisk (Text-fig. 1 D).

developed further and gave rise to a rather slow brown rot of the whole fruit.

From an examination of the specimens in Grove's herbarium, preserved at Kew, it seems likely that strain 3 corresponds to his interpretation of *Phoma solanicola* Prill. & Del. According to the original diagnosis this has spores $7.5 \times 3 \mu$. Köhler, who studied what he believed to be the same species, obtained spores from agar cultures with an average size of $5.99 \times 2.80 \mu$. The description of his cultures on malt agar agrees reasonably well with the appearance of group II though neither he nor Prillieux and Delacroix recorded the presence of septate spores. Köhler's measurements were mostly made on unextruded spores; for extruded spores, comparable to those measured by the writer, he obtained a higher value, viz. $8.64 \times 3.54 \mu$. Grove's collection from Wishaw has non-septate spores 6.0×2.5 (4.7×2.3) μ .

Strain 2 may well represent *Phoma tuberosa* of Melhus, Rosenbaum and Schultz (1916), described as having spores $3.7-6.1 \times 1.8-3.7\mu$ and as causing lesions very similar to those made by the fungi of group II. The original description of this species is quite inadequate, but the statements that the mycelial growth is 'whitish at first and at the end of a week begins to darken to the characteristic grey of *Phoma* spp.; the pycnidial development is very scarce. On potato cylinders the growth is fluffy and white, turning to grey within eight or nine days, the entire culture turning dark after the development of numerous pycnidia', is applicable to strain 2. *P. tuberosa* was originally described in 1914, but has subsequently been recorded from Maine (Anon. 1933), the Netherlands (van Poeteren, 1928), and, with some doubt, from England (Pethybridge, 1926). Unfortunately neither authentic material nor cultures of the American form could be obtained for comparison.

No fungus corresponding to strains 4 and 5 has been found in Grove's herbarium and it is doubtful what name he would have applied to it. It may possibly be the fungus listed on *Senecio* by Saccardo as *Phoma herbarum*, but this species is generally understood to have somewhat broader spores. Strain 6, on Vinca, may also have been included in *P. herbarum* if collected before. It can scarcely be *P. vincicola* Gonz., which has spores $10-12 \times 2\mu$, or *P. Kalkhoffii* Bub., with spores $5-7 \times 1\mu$. *P. linella* Desm. is said by Grove to be a *Phomopsis*. Strain 7 is presumably *Diplodina Sonchi* Henn. 1905; described with oblong-cylindrical, 1-septate spores $8-13 \times 3-3.5\mu$, on dry stems of *Sonchus asper*. Of all the above names, except *P. herbarum* which is of uncertain application, *P. solanicola* Prill. & Del. has priority. If *Phyllosticta Lonicerae* Westd. be accepted as the same species, however, it will take precedence as it dates from 1851. Much confusion exists as to the nomenclature of the *Phoma-Ascochyta* strains associated with foot rot of flax and much further study would be required before it can be decided how many of them are actually distinct. A disease apparently identical with that associated with strains 8 and 9 was described in Ireland as due to a *Phoma* sp. (Pethybridge, Lafferty & Rhynehart, 1921). According to Rost (1938) the fungus causing a similar disease in Germany is *P. lini* Pass. with spores $3-7 \times 1.5-3\mu$. In his view this is distinct from *P. linicola* Marchal & Verplancke to which Pethybridge, Lafferty and Rhynehart's fungus has been referred. Rost also claimed that a strain of *P. lingam* destroyed flax seed sown in soil inoculated with it.

Clearly strains 8 and 9 represent a single fungus, differing merely in the relative proportions of one- and two-celled spores in the pycnidium at the moment of examination. According to the current classification they must be placed in *Ascochyta* or *Diplodina* and seem to agree best with the description of *Ascochyta linicola* Naumoff & Vassilievski. Inoculation experiments with flax carried out by Foister after my transfer to Kew have shown that of all the strains tested only those isolated from flax could attack flax. Evidently strains 8 and 9 represent a species distinct from the other fungi in group II.

Group III. This group contains only *Diplodina Lycopersici* Hollos, which has been repeatedly described by previous workers. For comparison with

group II a brief account of its growth on my medium is given here. The mat is at first sparse and white, with tangential dark olive-green patches among the submerged hyphae by the fourth day. By the eighth day the mat is predominantly dark olive-grey, short and close, passing radially into a short white mealy growth which fades imperceptibly into the extending margin. From below the central 2 cm. already appear almost solid black. The rest of the submerged hyphae appear olive-grey, dotted with pycnidial rudiments in rather ill-defined concentric zones. The margin is whitish with a hint of zoning.

On the whole the mat is less dense and more even than that of group II and has a hint of brown towards the centre. The margin is less crenate and the pycnidial rudiments more abundant; towards the edge of the plate they are usually conspicuously elongated and deformed. No pycnidia matured in agar cultures (Text-fig. 1 J). On sterilized potato plugs the growth is quite unlike that of strain 2. There is a very sparse aerial mat and a densely crowded layer of pycnidia, making the surface look brown, rather than grey. The sterile 'stilboid' bodies figured by Wollenweber and Hochapfel (1936) were abundant when the cultures were about three weeks old.

The spores of strain 11 were about 24 % septate in pycnidia developed on tomato stems inoculated 29 March and examined 25 May, after being kept six days in a moist Petri dish. The unicellular spores measured $4.7-9.0 \times 3.3-4.5 \mu$, average $6.4 \times 4.1 \mu$, and the 2-celled ones $7.6-11.6 \times 4.2-5.3 \mu$, average $9.4 \times 4.7 \mu$.

In addition to the small differences in appearance of the mat in culture group III differs from group II in the presence of the 'stilboid' bodies, in the different reaction on potato tubers and in its more aggressive behaviour on living tomato stems. 10 % gelatine was liquefied by it to the depth of 5 and 16 mm. in fifteen and thirty days respectively. This fungus was regarded by Grove (1935) as identical with *Phoma destructiva* Plowright emend. Jamieson. This is improbable, for all workers who have studied the two fungi in culture regard them as distinct. Klebahn (1921) differentiated them on the ground that *Diplodina Lycopersici* did not mature pycnidia in agar culture while *Phoma destructiva* did so readily, but produced no septate spores. Brooks and Searle (1921) were similarly able to distinguish between two series of cultures, one of which they regarded as *P. destructiva*, the other as *D. Lycopersici*. Wollenweber and Hochapfel (1936) similarly conclude that *P. destructiva* 'Von *Diplodina* = *Ascochyta Lycopersici* unterscheidet sich der Pilz durch die kleinern, fast ausschliesslich einzelligen Sporen und sein durchscheinend auf die Tomatenfrucht beschränkte Schadwirkung'. I was unfortunately unable to obtain an authentic culture of *Phoma destructiva* as understood by Jamieson (1915), but it is worth noting that in her experiments the fungus would not attack potato tubers whereas *Diplodina Lycopersici* does so readily. The occurrence of what he believed to be *D. Lycopersici* in potato stems in the field has been reported from Jersey by Small (1936). It may be noted, however, that the rotting of tomato fruit and production of lesions when inoculated to tomato stems are not sufficient criteria for identification of *D. Lycopersici*.

Group IV. This group contains only strain 12 from living ivy leaves.

There is at first rather sparse white aerial growth in culture, with no dark hyphae, but by the fourth day the margin of the mat is already becoming irregular. By the sixteenth day the mat has changed to a rather sparse grey growth with whitish tips. The edge is almost free from aerial mycelium, white and crenate. The submerged hyphae are chocolate coloured to blackish brown over the greater part of the culture, but only form a solid mass of colour over the central 2 cm. The mature plate six weeks old is distinguishable by its short dark grey-brown growth matting down in a reticulated fashion with only occasional paler tinges. The submerged olive-brown hyphae are still rather sparse towards the margin, but fairly evenly developed, with irregular darker and lighter zones over the central 6 cm. No pycnidia developed in plate cultures, but they are plentiful on agar slants one month old (Text-fig. 1 H). No exudate was seen, but spores obtained by crushing apparently mature pycnidia were 4.7×2.3 ($3.0-6.2 \times 1.9-2.8$) μ (Text-fig. 3 E). On the host the spores measured 5.6×2.5 ($4.7-6.2 \times 2.2-3.1$) μ , but were not very abundant.

Strain 12 liquefied 10 % gelatine to a depth of 14 and 21 mm. in fifteen and thirty days respectively. It induced no lesions when inoculated into potato tubers, swedes, tomato stems and fruit or apple fruit.

The original collection was on living leaves marked with grey spots with dark-brown indistinctly zoned margins and would undoubtedly be called *Phyllosticta hedericola* Dur. & Mont. It is noteworthy, however, that Grimes *et al.* (1932) obtained from similar lesions a fungus identified as *Phoma destructiva* which rotted tomato fruit quite readily. It would appear, therefore, that lesions of the type known as *Phyllosticta hedericola* are associated with more than one fungus.

Group V. Strain 13, isolated from a pycnidium on a fallen ash fruit, forms a rather loose white aerial mat, shorter towards the edge, but without a clearly differentiated extending margin. Growth was slower than in most strains studied and at the end of four days there was only a dense white dome of hyphae. By the eighth day there was a dense white mat, up to 5 mm. thick, somewhat loose on the surface and turning light grey-brown over the central portion. From below the central area was seen to be mottled all over with shades of chestnut brown, leaving a sharply defined uneven white margin 2-3 mm. wide. After a fortnight growth had ceased. There was a finely lobed narrow white margin to a dense yellow-brown mat mottled brown below as on the eighth day. The marginal lobes were rounded, not dendritic as in group II. Older cultures differed only in the presence of white tufts of aerial mycelium, especially towards the margin. Pycnidia were not formed in any plate culture, but matured with pink exudate in 14 days round the holes in an agar slant from which inoculum had been taken (Text-fig. 1 M).

On oatmeal-agar strain 13 attained a diameter of 44 mm. in twelve days, forming a pure white, close mat, light grey only over the central 15 mm. At this time it was still colourless below except for a grey-brown tint in the central 2 cm. When five weeks old the plate was covered with a close white aerial mat beginning to collapse without further discoloration. Blackish brown submerged hyphae occupied only the central 2 cm., apart from

a narrow irregular zone of about 65 mm. diameter, apparently marking a stage at which growth had been temporarily checked. There were no pycnidia and there was no hint of red in the culture.

The submerged hyphae from malt agar cultures were light brown, with no tinge of olive under the microscope, mostly about 6μ wide, swelling occasionally to 8μ , and composed of elongated cells with few oil globules. There were no chlamydospores.

On tomato stems the pycnidia were about 200μ across, distinctly ostiolate, but without necks. The hyphae in the surrounding tissue were up to 10μ broad, closely septate and hyaline. Spores from these pycnidia were 7.7×4.0 ($5.6-10.4 \times 3.3-4.2$) μ elliptical to subcylindrical and non-guttulate. Occasional 1-septate spores measured about $10.4 \times 4.8\mu$. In the pink exudate from pycnidia on a malt agar slant the spores were biguttulate 6.8×3.7 ($5.9-8.7 \times 3.1-4.2$) μ and septate spores were not seen (Text-fig. 3 E).

On Doon Star tubers strain 13 formed lesions 4.5 mm. across (2-7 mm.) in eight out of ten inoculations and similar small localized lesions on swede roots. Of three inoculations to living tomato stems one failed, the others yielded lesions which attained a maximum size of 9×2 and 9×4 mm. respectively and bore a few mature pycnidia. The fungus rotted green tomato fruit, giving lesions 11 and 27 mm. across in seven and fifteen days respectively. On apple the lesions did not exceed 5 mm. 10 % gelatine was liquefied to a depth of 7 and 13.5 mm. in fifteen and thirty days respectively.

This fungus cannot be *Phoma samararum* Desm., for according to Wollenweber and Hochapfel (1936) that species is a *Septoria* with 0-septate spores $7-12 \times 1.7-2.2\mu$ and 1-septate spores $10-15 \times 1.7-2.2\mu$. It appears, however, to resemble closely their description of *P. aceris-negundinis* Arcangeli which, in turn, differs only slightly from *P. striaeformis* Dur. & Mont. isolated by them from very diverse hosts. The latter is said to include among its synonyms *P. Bismarckii* Kidd & Beaumont, described from rotting apples in England.

Group VI. Included in this group are strains 14-16 inclusive. The following description of growth in culture is based primarily on the behaviour of strain 15.

After four days there is a rather loose white mat rising gently from the margin to the centre of the mat. By the eighth day the very short white mat has developed small depressions on its surface and has a hint of greyness at the centre. There is diffuse grey mottling of the submerged growth in the central 20 mm. On the fifteenth day the mat is still white, faintly grey at the centre, but is closely pitted all over in a very characteristic fashion so that the whole surface of the culture appears reticulated. By this time the submerged mycelium has become light olive-grey over the central 25 mm. and pycnidia are plentiful, bearing salmon-pink exudate. As a rule they tend to be arranged in radiating rows instead of in concentric zones, as though due to sectoring. After a month's growth there is no change in the general appearance except that an olive-brown tint has spread over the greater part of the submerged growth (Pl. II, fig. 8).

Strain 14 (Text-fig. 1 N) differed only in the submerged mycelium

turning olive-grey only from the fifteenth day onwards. Strain 16 appeared identical with strain 15.

The spore measurements of these three strains are given in Table 5, with the gelatine liquefaction data for strains 14 and 15. Strain 16 was not grown on gelatine.

Table 5. *Spore measurements of strains 14, 15 and 16*

Strain No.	Substrate	Unicellular spores	2-celled spores	Depth of gelatine liquefied in	
				15 days	30 days
14	Malt agar plate 1 month old	7.4×3.4 ($5.9-9.6 \times 2.5-4.2$) μ	11.2×4.2 μ	0	15 mm.
15	Malt agar plate 18 days old	7.2×3.4 ($5.0-10.1 \times 2.8-4.2$) μ	11.2×5.0 μ	Trace 7 mm.	
	Stem of <i>Centaurea Scabiosa</i>	7.0×4.0 ($6.2-7.9 \times 3.4-4.5$) μ	—		
16	Malt agar plate 7 days old	7.6×3.4 ($5.9-9.3 \times 2.8-3.9$) μ	11.9×5.8 ($9.8-14.7 \times 4.8-6.5$) μ	Not tested	
	Living leaves of <i>Ribes Grossularia</i>	6.3×3.2 ($5.0-7.6 \times 2.5-3.6$) μ	—		

The spores are always broadly elliptical, biguttulate or with groups of polar granules (Text-fig. 3 E). Septate spores were very few in the exudate of strains 14 and 15, barely 1 % in strain 16. Occasional rounded chlamydospores about 20μ across occurred in the submerged hyphae of strains 14 and 15.

Strains 14 and 15 induced no lesions on potato tubers, tomato stems or fruit, swede roots or apple fruit. Strain 16 gave similarly negative results on potato, tomato and apple fruit, but was not tested on swedes or tomato stems.

Strain 16 would certainly be classed as *Phyllosticta Grossulariae* Sacc. and there seems every reason to regard strains 14 and 15 as identical with it, though a fungus corresponding to strain 15 was apparently included by Saccardo under *Phoma herbarum* West. Strain 14 was obtained from immature pycnidia without spores on a fallen fruit of *Fraxinus excelsior*.

Group VII. This includes strains 17 and 18, both isolated from the tissues of potato tubers showing superficial necrosis.

On malt-agar plates there is at first a dense white mealy growth, below which olive-coloured hyphae are already apparent by the third day. By the fourth day there is a distinctly differentiated marginal zone white below, with very sparse whitish aerial growth above, about 4 mm. wide. Within this is a dense taller grey mat, dark olive-grey below. A mature culture shows a rather short dense mealy olive-grey mat, paling towards the margin where it passes into a narrow pale extending zone only 1-2 mm. wide (Pl. II, fig. 1). Such a culture appears black at the centre below, passing into olive-grey towards the margin.

On sterilized potato plugs there is a dense dark grey growth, olive-coloured at the dry upper end. A crust of pycnidia forms on the surface of the tissue within three weeks from inoculation and exudes a copious grey slime of conidia.

Pycnidia did not appear in the original mycelial transplants on malt agar, but when a brush streak was made on a malt agar plate with a suspension of conidia from a potato plug culture a crowded band of pycnidia developed along the streak (Text-fig. 1 L). Below these the medium appeared black owing to the submerged hyphae and chlamydospores, but on each side of the streak remained a clear band, each $3\text{--}4\mu$ wide and 8 mm. apart. The rest of the plate became covered with the normal mat described above.

Both fungi produced abundant and characteristic chlamydospores, mostly in pairs or short chains, and measuring individually about $7\text{--}13 \times 5\text{--}10\mu$ (Text-fig. 3 D). In some instances these chlamydospores are produced in such numbers on infected potato tubers as to give rise to a coal-black layer immediately under the skin.

Strain 18 was received as a single spore culture from Dr A. R. Wilson. Single spore isolations of strain 17 made from the potato plug culture did not differ in appearance from the original mycelial isolate.

Inoculation experiments showed both strains to be non-parasitic to all the experimental hosts with the possible exception of apple fruit, on which minute round lesions $3\text{--}4$ mm. in diameter developed with both fungi. Strain 18 had been identified by Dr Bisby as *P. eupyrena* Sacc. as interpreted by Wollenweber, and it is interesting to note that the latter reported his isolate to cause rotting of apple, cucumber and potato. It is curious, however, that Wollenweber and Hochapfel, writing subsequently, did not mention *P. eupyrena* among the *Phoma* species causing minor rots of fruit. Wollenweber's original isolate was regarded by him as the cause of blackish pimples on potato skins (Wollenweber, 1920). As Shapovalov (1923) has shown, however, these were obviously the disease now known as Skin Spot and the *Phoma* must have been a secondary invader. Shapovalov, however, contributed to the confusion by confounding Skin Spot with the early stages of Powdery Scab. Exuded pycnosporos of strain 17 on potato plugs measured $3.4\text{--}5.0 \times 2.2\text{--}3.6\mu$, mean $4.3 \times 2.5\mu$ (Text-fig. 3 E). Those obtained by crushing pycnidia developed on the host were, as usual, somewhat smaller, with mean size $3.1 \times 1.9\mu$. Septate spores were not observed. On 10% gelatine strains 17 and 18 behaved exactly alike, liquefying 6.5 and 12 mm. in fifteen and thirty days respectively. The identity of Wollenweber's fungus, with spores $4\text{--}6 \times 1.7\text{--}2.3\mu$, with Saccardo's which had spores $4 \times 1.5\mu$ seems open to question.

Group VIII. This contains only strain 19, but an identical fungus was isolated from similar pycnidia on *Anthriscus sylvestris* in Corstorphine.

In Petri dish cultures this strain formed a close, short pure white mat with a mealy surface (Pl. II, fig. 2). There was no zoning and no discoloration of the aerial mycelium until it began to degenerate and turn light grey when about one month old. The marginal extending zone was very narrow and passed imperceptibly into the main body of the mat. The submerged mycelium showed a faint greenish brown hue at the centre on the seventh day and the colour slowly darkened to olive-grey and ultimately black at the centre. Ill-defined concentric lighter and darker zones were apparent by the eighteenth day. Pycnidia, at first yellow-brown,

ultimately black, appeared in the central area when about a week old (Text-fig. 1 K). There were no chlamydospores.

Spores from the original pycnidium on *Heracleum* measured $7.0-10.0 \times 2.5-3.5 \mu$; those from the pink exudate of pycnidia on a two months old Petri dish culture measured $7.8-11.6 \times 2.5-3.6 \mu$, mean $10.3 \times 3.2 \mu$, with occasional two-celled spores $11.2 \times 4.2 \mu$ (Text-fig. 3 E).

Strain 19 induced no lesions on any of the experimental hosts. This fungus is obviously *Phoma complanata* Desm. and is distinguished from all the preceding groups by the massive character of its pycnidia, the wall of which in culture consisted of about 6 layers of cells instead of 2-3 layers.

Group IX. Group IX contains five strains, numbers 20-24 inclusive, all characterized by massive black pycnidia, small cylindrical spores, cream-coloured exudate and slow-growing close grey mycelial mats in culture. The following description applies to strain 20; the mat produced by the other strains differed in detail as indicated below.

Table 6

Strain No.	Substrate	Spore size	Depth of gelatine liquefied in	
			15 days	30 days
20	Dead stem of <i>Urtica dioica</i>	4.7×1.8 ($3.9-5.4 \times 1.6-1.9$) μ	7 mm.	14 mm.
	Malt-agar slant 12 weeks old	4.3×1.5 ($3.6-4.8 \times 1.4-1.9$) μ		
21	Dead stem of <i>Sisymbrium officinale</i>	4.6×1.5 ($4.0-5.3 \times 1.4-1.6$) μ	Trace	15 mm.
	Malt-agar plate 8 weeks old	5.1×1.5 ($4.2-5.6 \times 1.1-2.2$) μ		
22	Dead umbelliferous stem	4.5×1.8 ($3.6-5.4 \times 1.6-2.0$) μ	Trace	15 mm.
	Malt-agar plate 4 weeks old	5.2×1.7 ($4.2-6.2 \times 1.4-2.5$) μ		
23	Dead stem of <i>Lapsana communis</i>	5.0×1.7 ($4.5-5.5 \times 1.5-2.0$) μ	5 mm.	19 mm.
	Malt-agar plate 4 weeks old	5.1×1.5 ($4.2-6.7 \times 1.1-2.2$) μ		
24	Dead stem of <i>Anthriscus sylvestris</i>	5.5×2.1 ($4.5-6.8 \times 1.6-2.5$) μ	Trace	14½ mm.
	Malt-agar slant	4.7×1.6 ($3.6-5.9 \times 1.4-2.0$) μ		

By the seventh day there was a dense grey mound of aerial mycelium, about 3 mm. thick, over the inoculum, surrounded by a short, close, olive-grey mat with a very narrow, paler, extending zone (Pl. II, figs. 4-7). The culture already appeared an even olive-grey colour from below. This appearance was maintained throughout the life of the culture except that the mat tended to become somewhat mealy due to the presence of closely crowded short grey tufts on a darker basal layer of hyphae. No chlamydospores were seen, but ripe pycnidia were present by the eighteenth day. Very few were produced, however, and in many subsequent agar slant cultures none was found.

Strain 21 differed in showing distinct colour zoning in the submerged growth and in producing more pycnidia. Strains 22 and 23 were similar to 21, but had numerous pycnidia in concentric zones; number 22 had more and taller aerial mycelium and less colour in the submerged hyphae than 23 (Pl. II, fig. 9). Strain 24 was intermediate in appearance between 22 and 23, rather slower growing than either, and bore no pycnidia on the Petri-dish cultures. The spore dimensions and gelatine liquefying capacity of the five strains are set out in Table 6.

Septate spores were not observed in any strain of this group (Text-fig. 3 E).

Strain 24 was isolated from a pycnidium which formed one of a group round which the surface of the host was stained a faint pink colour (Text-fig. 2 B). No pink pigment was produced by this strain in culture, either in the hyphae or in the medium and the association with staining of the host was probably fortuitous.

The original collection of strain 22 was characterized by very long beaks to the pycnidia (Text-fig. 2 C). This character was retained in culture, where it was exhibited to an equal extent by strain 24 (Text-fig. 2 E, F). The remaining strains produced pycnidia with distinct but usually shorter beaks (Text-fig. 2 G). All strains gave uniformly negative results in inoculation experiments with all the differential hosts.

Strain 20 is *Phoma acuta* Fekl. (Text-fig. 2 D). The remaining strains are clearly closely allied to it and there seems little justification for attempting to separate strains 21 and 23 on morphological or other grounds. The two strains from Umbelliferae may possibly be differentiated on a basis of pycnidial shape, though this seems to be a somewhat variable and unreliable character. On the whole it seems best to regard all five fungi as representing a single species widespread in nature on dead herbaceous stems. Strain 23 may be *Phoma Lampsanae* Karst., but it is doubtful if numbers 21, 22 and 24 have been described as distinct species. Number 24 in general appearance suggests *P. sanguinolenta* Grove, but has smaller, narrower spores.

Group X. Strains 25-27 inclusive are all easily recognizable in culture by their abundant pycnidia and sparse aerial mycelium. In spite of some variation in size and number of pycnidia they appear to form a natural group. The following description applies primarily to an early transfer of strain 25. By the fourth day the culture had attained a diameter of 15 mm. with no aerial hyphae, but was already covered with pycnidia. On the seventh day there was a little aerial mycelium, scarcely visible to the naked eye. The whole surface of the culture was covered with yellow-brown pycnidia (Pl. II, fig. 3). On the ninth day a few short white aerial tufts were present. The central portion of the growth was covered by a yellow-brown crust of pycnidia; surrounding this was a zone of less crowded darker pycnidia followed by a marginal area 8 mm. wide in which fructifications had not yet developed. There were no dark submerged hyphae and the only changes in appearance of the culture with age were more pronounced zoning in density of the pycnidia, change in their colour to dark brown with a pinkish ooze of spores and more abundant clumps of white aerial mycelium which ultimately turned grey-brown.

Strain 26 looked identical except that the pycnidia appeared slightly smaller (Text-fig. 1 R; Pl. II, fig. 10). No chlamydospores were found in either fungus.

As shown by the photographs these early transfers very closely resembled *P. hibernica* Grimes *et al.* (1932) and a culture of strain 26 sent to Prof. Grimes was accepted by him as being probably that species.

Subsequent transfers of numbers 25 and 26 differed from the above description, however, in producing fewer pycnidia and more white aerial

mycelium. Moreover, old plates developed a certain amount of submerged olive-brown mycelium. Hence when grown on the same plates with *P. hibernica* they appeared quite distinct from it. Presumably this change was due to staling. A fresh isolate of strain 25, inoculated with *P. hibernica* to opposite sides of the same Petri dish, showed the two to grow at exactly the same rate and to appear alike except that this time number 25 produced far fewer pycnidia (Pl. III, fig. 1). Hence the general aspect of the culture was whitish whereas that of *P. hibernica* appeared pink at the centre from the extended conidia and yellow brown towards the margin owing to the crowded immature pycnidia. Older Petri-dish cultures of *P. hibernica* developed towards the margin extensive sectors carrying few pycnidia and with considerable light grey-brown aerial growth. Obviously there can be a great deal of variation in the relative proportions of pycnidia and aerial mycelium and it seems unwise to attempt to separate the three strains on this character alone. Spore sizes are indicated in Table 7.

Table 7. *Spore sizes and gelatine liquefaction by strains 25-27*

Strain no.	Substrate	Spore dimensions	Depth of gelatine liquefied in	
			15 days	30 days
25	Dead stem of <i>Urtica dioica</i>	5.1×2.0 ($3.3-5.9 \times 1.6-2.2$) μ	10 mm.	19 mm.
	Malt-agar plate 2 months old	5.8×2.9 ($4.5-6.8 \times 2.2-3.9$) μ		
	Malt-agar slant 14 days old*	5.8×3.1 ($4.7-6.8 \times 2.8-3.6$) μ		
26	Dead stem of <i>Angelica sylvestris</i>	6.6×2.8 ($5.0-7.3 \times 2.0-3.1$) μ	18 mm.	30 mm.
	Malt-agar plate 5 weeks old	6.4×3.2 ($5.3-7.6 \times 2.5-4.2$) μ		
27	Malt-agar slant 14 days old	7.5×3.7 ($6.7-9.3 \times 3.3-4.5$) μ	10 mm.	17 mm.
	As cited by Grimes <i>et al.</i>	5.7×4.4		

* These figures apply to spores of 'normal' elliptical shape. About 25% of those in the exudate on this particular slant were irregular or semiglobose and measured about 6.8×6.0 ($5.9-7.6 \times 5.3-6.8$) μ (Text-fig. 3 E).

All three strains were completely non-pathogenic to all the differential hosts.

Strain 25 seems to be *Phoma Urticae* Sch. & Sacc. Strain 26 probably corresponds to five collections of a *Phoma* on *Angelica* in the Grove herbarium, placed by him under *Phoma oleracea* Sacc. It has, however, slightly larger spores than the pycnidia examined from Grove's material, which had spores $4.5 \times 1.5-2.2 \mu$. Whether *P. hibernica* is identical with either or both the other strains of this group is rather doubtful. The culture received from Grimes under that name had larger spores than either numbers 25 or 26. On the other hand, the diagnosis of *P. hibernica* states it to have spores $5.7 \times 4.4 \mu$, i.e. distinctly shorter than those measured from strain 27. Bisby *et al.* (1933), on the other hand, obtained a strain of this type with narrower spores, $4.7 \times 2.3 \mu$. It would appear that the spore size is not constant and that much weight cannot be placed on the differences brought out in Table 7.

Prof. Grimes kindly suggested comparative cultures on a special medium to settle the point, but as the medium included much cream it could not be used during the war. The relationship of *P. hibernica* to other species can scarcely be regarded as determined until the source from which it con-

taminates cream and milk products is discovered. So far, the species has been reported from two localities. It is evidently common at Cork in milk, cream, butter and water, and Bisby *et al.* (1933) encountered it in butter in Manitoba.

Group XI. Phoma nebulosa, as represented by strain 28, is not uncommon on the upper part of dead *Urtica dioica* stems (Text-fig. 2 N) and usually occurs side by side with *P. Urticae*. Owing to the tendency of the latter to vary in culture as regards the quantity of aerial mycelium one is tempted to regard *P. nebulosa* as merely the extreme mycelial form of group X. Its behaviour in culture, however, is so constant that the suggestion seems unwarranted. There was no possibility of confusing the most extreme mycelial phase of strain 25 or 26 with strain 28, owing to the presence in the latter of dark submerged mycelium from the outset.

By the fifth day a culture of the latter consists of a dense white mass of aerial mycelium covering the central 2 cm., with sparse hyphal development on the remainder of the mat. Olive-brown submerged mycelium was already present over the central 1 cm. By the eighth day almost the whole culture was covered with a dense woolly white growth, sparse over the marginal centimetre and fading into the extending edge which was already crenate (Pl. III, fig. 2). Scattered pycnidial rudiments were visible towards the centre of the mat. Further growth led to little change in appearance except that the submerged hyphae became dark brown at the centre with a few irregular lighter and darker concentric zones towards the margin. On a mature Petri-dish culture the woolly aerial growth, continuous at the centre, tended to become broken up into islands towards the margin, so that the dark submerged hyphae were visible between them.

On sterilized potato plugs there was the same dense pure white growth covering a layer of chocolate to dark grey-brown mycelium on the surface of the plug and along the lines of contact with the glass.

No pycnidia matured either in Petri-dish cultures or on agar slants, but they were very abundant on the potato plugs within 14 days of inoculation (Text-fig. 2 O).

The pycnidium from which strain 28 was isolated contained rather irregularly cylindrical spores with rounded ends, occasionally pointed at one end and sometimes three-cornered in shape (Text-fig. 3 E). The non-septate spores measured 9.3×3.7 ($7.6-11.3 \times 2.8-4.5$) μ . Among these were about 2 or 3 % of septate spores measuring 11.4×4.3 ($9.3-13.2 \times 3.6-5.0$) μ . Spores crushed from pycnidia in a potato plug culture fifteen days old were cylindrical with rounded, often slightly swollen ends, mostly biguttulate, 9.3×3.4 ($7.5-11.3 \times 2.8-3.9$) μ . Other pycnidia on the same piece of discoloured nettle stem contained non-septate spores up to $11 \times 2 \mu$. Isolations from these yielded the same type of culture as strain 28.

P. nebulosa liquefied 10 % gelatine to the extent of 11 mm. and 24 mm. in fifteen and thirty days respectively. It induced no lesions when inoculated to any of the experimental hosts.

Group XII. This contains only strain 29 isolated from a pycnidium on a dead figwort stem. By the third day there was already dark olive-grey submerged mycelium at the centre of the culture, covered by a close short

white mealy growth. On the eighth day there was a hint of greyness at the centre of the aerial mat with vague zoning towards the margin (Pl. III, fig. 8). A few scattered pycnidia were present, but the dark colour had not extended in the submerged mycelium. By the twelfth day, however, greyish zone lines were apparent below and pycnidia were scattered over most of the culture. After seven weeks the aerial growth was still white, though beginning to break down. Pycnidia were plentiful over the central 50 mm., though more numerous in two zones. Outside this area were two more scattered belts of pycnidia. Slight development of dark submerged hyphae had taken place around the pycnidia, but not enough to discolour the lower surface as a whole. Pycnidia of this strain in culture tended to occur fused together to form large, irregular, multilocular masses more or less conical in shape and from 1 to 2 mm. across (Text-fig. 3 B). The exudate was pale pink. Spores from a month old culture measured 6.0×2.0 ($4.7-7.3 \times 1.4-2.5$) μ (Text-fig. 3 E). Those on the host were rather narrowly cylindrical, with rounded ends, sometimes slightly curved, biguttulate 5.5×2.1 ($4.5-6.5 \times 1.9-2.5$) μ . The fungus was not parasitic to any of the usual hosts. It liquefied 10% gelatine to a depth of 12 mm. and 26 mm. in fifteen and thirty days respectively. This strain is evidently a form of *Phoma oleracea* Sacc. and five similar collections on *Scrophularia* occur in the Grove herbarium under that name or as var. *Scrophulariae* Sacc.

Group XIII. Strain 30, from a dead goldenrod stem, has spores rather similar to those of group IX, but appears sufficiently different in culture to warrant its retention as a separate group. Also the pycnidia, both on the host and in culture, were quite unlike those of *P. acuta* (Text-fig. 2 H, J). They were far less massive and on the goldenrod stem superficially resembled those of group XII on *Scrophularia*, but with thinner walls (cf. Text-fig. 3A).

The aerial mat was at first dense, pure white, with an evenly rounded surface, but by the fifth day the centre was turning grey and a dark olive-grey hue with darker spots had spread over the middle 2 cm. of the submerged mycelium. By the eighth day the culture was easily distinguished from those of any other strain by its dense woolly aerial mat, the same grey as strain 20, but forming a very flat cone with the margin paling into white (Pl. III, fig. 4). Almost the entire lower surface of the culture appeared dark olive-grey, with a narrow, rather sharply defined, white margin. There were no pycnidia on Petri-dish or agar-slant cultures.

On potato plugs there was the same light grey woolly growth, almost white in places, covering an olive-grey layer which appeared almost black along the lines of contact with the glass. Small pycnidia were very abundant after about a fortnight's growth (Text-fig. 2 J).

The spores of the original collection were $5.7 \times 2.0-2.5 \mu$; those on the sterilized potato tissue were 5.5×1.5 ($4.2-6.2 \times 1.0-2.5$) μ (Text-fig. 3 E). 10% gelatine was liquefied to a depth of 10 mm. and 19 mm. in 15 and 30 days respectively. The following appear to be the only *Phoma* spp. recorded on *Solidago*:

P. herbarum West f. *Solidaginis* Sacc. with spores $7-9 \times 3-4 \mu$.

P. Solidaginis Cke, with spores $9-10 \times 2 \mu$.

Neither of these appears to agree well with strain 30. There is also a *Phyllosticta Solidaginis* Bres. with spores $5-6 \times 2 \mu$, but it is idle to speculate on a possible relationship with such a form. Strain 30 cannot therefore be named at present.

Group XIV. The fungus from goose-grass bore some resemblance in culture to Group I, but is easily distinguished from it by other characters. Growth was at first thin, sparse and white. After one week the aerial mycelium was still very thin, but there was an olive-brown tinge to the central 2 cm. of the lower side of the culture. By the twelfth day the whitish marginal zone was already somewhat crenate and surrounded a rather thin, short, chocolate-coloured aerial mat. The submerged mycelium was olive-brown to almost black and formed a fibrous radiating network, not a solid mass of colour (Pl. I, fig. 3). By the eighteenth day there was irregular wavy zoning very like that of Group I.

The submerged hyphae were rather broad, mostly $5-9 \mu$ across, dark brown and crowded with oil globules as in *P. foveata*. No chlamydospores were seen. Pycnidia were scattered rather sparsely over the culture and produced a whitish exudate when mature. On the host the spores were cylindrical or slightly curved, with rounded ends, mostly biguttulate, and 8.5×3.1 ($7-9.8 \times 2.8-3.4$) μ in size. As in strain 25 the spores exuded from pycnidia in culture tended to be irregular and swollen and measured 5.8×3.8 ($4.5-7.6 \times 3.1-4.8$) μ (Text-fig. 3 E). Strain 31 liquefied gelatine to the extent of 13 mm. and 24 mm. in fifteen and thirty days respectively.

From *P. foveata* this strain differs in its somewhat larger spores, different coloured exudate and inability to produce lesions on potato, swede, tomato stems or fruit. Minute circular lesions were produced on apple fruit, but no rot developed. It is rather difficult to decide what to call this strain. The material labelled *P. herbarum* f. *Galiorum* Sacc. in Grove's herbarium, though superficially similar, has quite different elliptical spores, mostly $4-5 \times 2-3 \mu$. When re-examined nine months after collection the Berwickshire material showed a few of the larger spores to have become 1-septate. Hence the fungus is probably the same as that identified by Grove with *Diplodina Gallii* (Niessl) Sacc., collected on *G. Mollugo*, Polperro, Cornwall, with spores $8-10 \times 3 \mu$, though almost all the spores of that collection are now distinctly septate.

Group XV. Strain 32, from a pycnidium on gooseberry twigs, differed from all other strains encountered in the small size of its spores. Growth in malt-agar culture was extremely slow, not exceeding 13 mm. in diameter in seven days. After fifteen days there was a dense, rather thick, olive-green mat passing laterally into a yellow-green zone and finally a rather thin narrow white margin. Fine whitish tufts of hyphae were scattered throughout (Pl. III, fig. 7). The lower surface was dark olive-grey at the centre, fading to an almost white margin. When one month old there was still a dense olive-grey dome of hyphae over the inoculum, surrounded by a narrow depressed zone. The remainder of the mat was about 2 mm. thick, light olive-grey and very close and even or with a finely reticulated surface. Towards the margin the colour changed to yellow-green. The very dark olive-grey hue of the submerged mycelium persisted at the centre, sur-

rounded by concentric zones of darker and lighter hue. A few black, globose, ostiolate pycnidia about 200μ across were scattered over the mat (Text-fig. 2 L). The pycnidial exudate was white. Submerged hyphae were composed of short dark olive-brown cells, but no distinct chlamydospores were seen. Strain 32 liquefied gelatine more slowly than any other, except perhaps strain 34, viz. to the extent of only $6\frac{1}{2}$ mm. in thirty days.

The spores on the host were rod-shaped, about $3 \times 1\mu$. In culture they measured 3.5×1 ($2.5-5.0 \times 1$) μ (Text-fig. 3 E). Strain 32 also differed from the others in having conidiophores considerably longer than the spores, up to about 16μ long. This suggests that it was a *Dendrophoma*, but branching of the conidiophores could not be detected. In some features it resembles Wollenweber and Hochapfel's description of *D. faginea* Ferraris which, however, apparently tends towards production of a brown colour in the lower side of the culture. Strain 32 was non-parasitic on potato, swede or tomato. On apple fruit it induced small circular lesions not exceeding 5 mm. in diameter.

Strain 32 is identical with the fungus preserved in Grove's herbarium under the name *Ascochyta Grossulariae* Died., on gooseberry tips from Lambourne Hill, West Cornwall, 17 April 1933, which has rod-shaped spores $3 \times 1\mu$ and conidiophores about 10μ long.

Group XVI. Strain 33 was clearly a form of *Phoma lingam* (Fr.) Desm., a species which has been repeatedly described in culture and need not be dealt with in detail here. Growth was at first dense, short, white and mealy, later turning grey above and dark olive-grey below. The pycnidial exudate was amethyst coloured and consisted of non-septate spores which measured 4.3×1.9 ($3.3-5.4 \times 1.6-2.2$) μ .

Gelatine liquefaction was unusually rapid, 24 mm. in fifteen days, though bacterial contamination could not be detected. Strain 33 produced no lesions on any host except swede, on which it gave rise to a rot, slow at first, but ultimately rapid, especially in the outer layers of the cortex.

Group XVII. Strain 34 was Brooks and Searle's original isolation of *Phoma alternariacearum*, kindly supplied by Prof. Brooks. This was originally isolated from a rotten tomato fruit purchased in Bristol in 1916 so that it had been maintained in culture for twenty-eight years. It is therefore not surprising that it appears to have lost its ability to cause a rot of tomato fruits. Growth in culture on malt agar corresponded fairly well with Brooks and Searle's description of its behaviour on modified Dox agar (Brooks & Searle, 1921, p. 181, Form C). After four days there was a short thin whitish mat already bearing abundant pycnidia (Text-fig. 2 M). By the eighth day the thin aerial growth was turning grey-brown. There were no dark submerged hyphae, but zoning was apparent in the arrangement of pycnidia. Older cultures showed no change in general appearance apart from the production of sectors with more or less numerous pycnidia (Pl. III, fig. 9).

The characteristic chlamydospores were noted in the aerial mycelium; they varied greatly in size, mainly from $20-50 \times 10-25\mu$ (Text-fig. 3 C). The pycnidial exudate was pink and contained ellipsoidal conidia, sometimes slightly pointed at one end, mostly biguttulate and measuring

6.2×2.9 ($5.0-7.3 \times 1.9-3.6$) μ (Text-fig. 3 E). This strain liquefied only 7 mm. of gelatine in 30 days.

According to Wollenweber and Hochapfel (1936) this is a synonym of *P. glomerata* (Corda) Wr. & Hochl.

DISCUSSION

The traditional allocation of pycnidial fungi to the form-genera *Phoma* or *Phyllosticta* and to *Diplodina* or *Ascochyta* according to whether they are collected on stems or leaves has long been recognized to be irrational. That it is retained is a tribute merely to its convenience and a confession of the necessity of grouping for easy reference the innumerable names applied in the literature to this group of Coelomycetes. In addition it is becoming evident that generic distinctions based on the presence or absence of a septum in the small spores of this group of fungi are not strictly valid. Thus Brooks and Seale (1921, p. 184) remark that 'In studying the various forms of group A [ultimately classified by them as *P. destructiva* and *D. Lycopersici*] it was found that these showed great variability in characters which are generally looked upon as specific, such as size of spores, septation of spores, guttulation and shape of spores, and one was forced to the conclusion that a number of so-called species in genera like *Phoma*, *Ascochyta* and *Diplodina* are nothing more than varieties of one and the same fungus.' Sattar (1934) similarly found that no sharp distinction could be drawn between Sphaeropsidales with hyaline continuous spores and those with 1-septate spores of similar shape, i.e. between the twin-genera *Phoma-Phyllosticta* and *Diplodina-Ascochyta*.

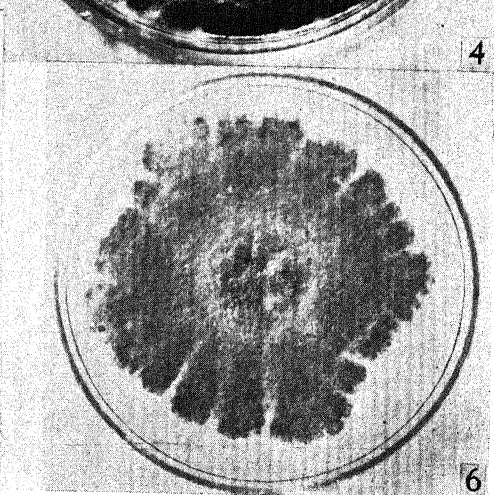
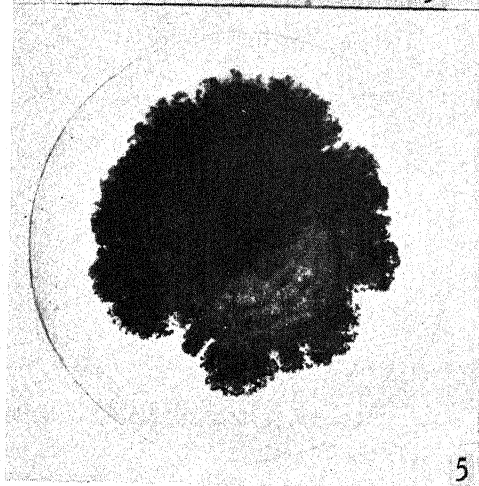
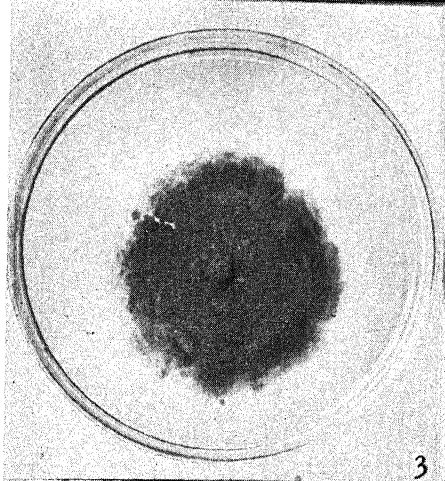
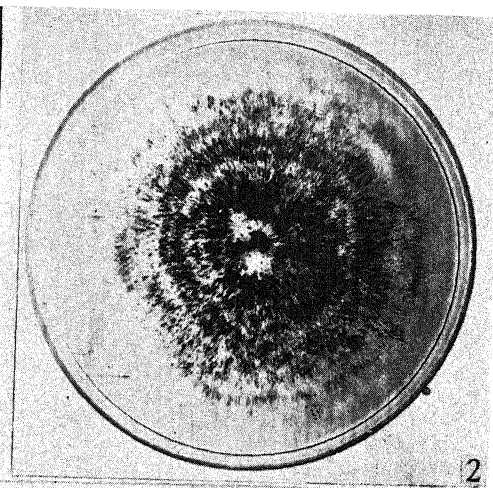
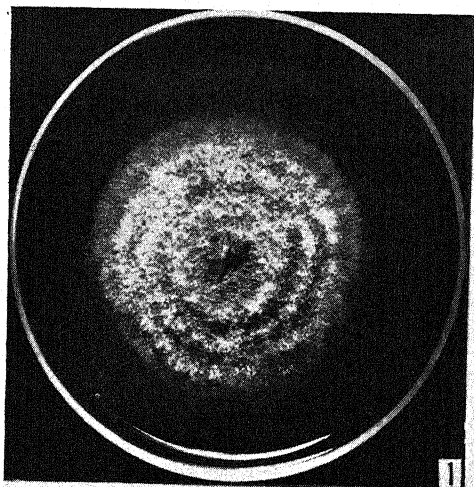
So many are the difficulties, however, in attempting to introduce some order among the vast number of host-species at present allotted to these genera that taxonomists are naturally reluctant to relinquish such a convenient generic character as the presence or absence of a septum in the spores. Thus Wollenweber and Hochapfel (1936), though admitting the tendency of most '*Phoma*' species to produce a few septate spores, attempted to define *Phoma* as having not more than 5 % of these. In their view 'The spores of many true *Phoma* species are not altogether one-celled, but there occur ± 5 per cent 1 (2-3) septate ones, as for example in *P. glomerata*, *P. prunicola*, *P. ellipticum*, *P. Mori*, *P. striaeformis*, occasionally also in *P. destructiva*, just as also in many other species not described here, such as *P. sarmentella* Sacc., *P. nebulosa* (Pers.) Berk. and *P. Libertianae* Speg. & Roum. Because they form a normal though sparsely represented component of the spore mass they must be taken into consideration in future descriptions of the genus *Phoma*. Fungi with generally about 50-100 per cent. of the spores septate are allotted to the *Hyalodidymae* (*Ascochyta*). Clearly this distinction is difficult to apply in practice, since it implies the absence of intermediate forms with from 5 to 50 % of the spores septate, though, as shown above in Group II, such forms are of common occurrence. Sattar (1934) found *Ascochyta pinodella* to have from 5 to 50 % of the spores bilocular on the host whilst in culture it produced only unicellular spores and hence was a *Phoma* or, as he calls it, a *Phyllosticta*. On the other hand, *Phyllosticta Rabiei*, which had at most 5-10 % of the spores bicellular, was

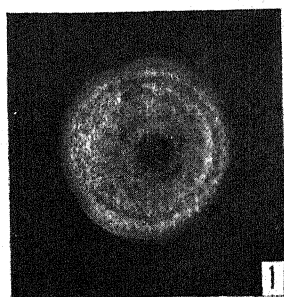
transferred by Sattar to *Ascochyta* because the spores became two-celled during germination. In attempting to solve the difficulty he concludes that 'one should, in my opinion, consider only spores obtained from the host'. Thus fungi like *P. hibernica*, only known in artificial cultures from contaminated butter and cream, could not be allocated to any genus, inasmuch as their host in nature is unknown. In practice it appears that a writer who has been working primarily with mainly continuous-spored species tends to ignore a considerable degree of septation in obviously related forms and allots them all to *Phoma*. Conversely a person interested in 'typical' species of *Ascochyta* seizes on the presence of an occasional septum in related species as an excuse to include them all in the same genus. Probably no great harm results from such procedure as long as it is generally recognized that the classification is merely a matter of convenience, and even of personal prejudice, and that a fungus cited in the literature under one generic name may have its closest allies listed under another. No more logical classification can be proposed until the species are better known. Before defining genera it is necessary to define the limits of the species they are to contain. It is in this connexion that the existing position is most alarming. The experiments reported above, though carried out with only thirty-four isolations, afford evidence that:

(1) The same fungus may be found in the present classification under several different names and probably in different genera; see for examples groups II and VI. Wollenweber and Hochapfel (1936) obtained even more striking evidence in this connexion. Thus under *Phoma seriata* (Pers.) Wollw. & Hoch. they cite fourteen synonyms in five genera, not including the obsolete '*Sphaeria*'. Under *Ascochyta pirina* (Fr.) Pegl. they cite three species of *Phoma*, one of *Diplodina* and one of *Clisosporium*.

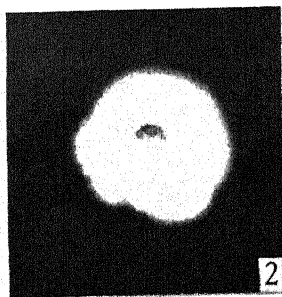
(2) The same name may be given to quite different fungi. This is not an unexpected discovery in the case of ill-defined species such as *P. herbarum* and *P. oleracea*, but it appears to be also the case with *Phyllosticta hedericola*. Grimes *et al.* (1932) have shown that one form of this host-species is indistinguishable from *Phoma destructiva*. The writer, on the other hand, has isolated under that name a fungus incapable of attacking tomato fruit, almost the only valid character, other than spore size, on which *P. destructiva* was founded.

(3) Fungi capable under favourable circumstances of parasitizing economic plants such as potato, tomato and apple may be found in nature as saprophytes on herbaceous weeds, where their presence has hitherto been unsuspected. Though the diseases in question are of minor significance, this discovery is of interest as indicating that, for example, gangrene-type lesions in potato may be expected to occur even when clean seed is planted in virgin soil. Clearly before plant pathologists apply names to minor parasites of this kind they must make an exhaustive study of the already described saprophytic species occurring in the same locality. What is urgently needed is a reinvestigation of the small-spored Sphaeropsidales, based on isolations from material matched with the existing host-species and subsequently grown for comparison under uniform conditions in artificial culture and on suitable differential hosts.

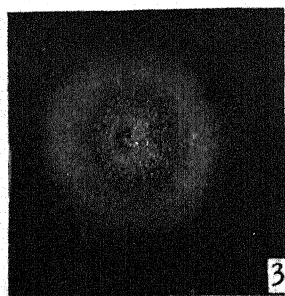




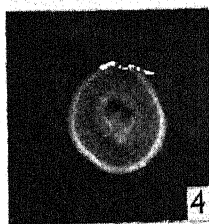
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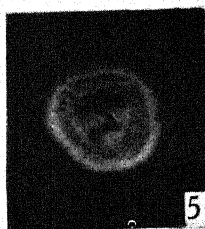
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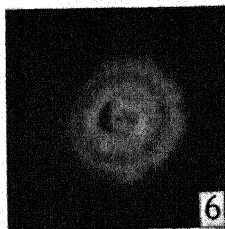
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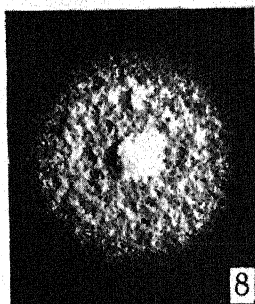
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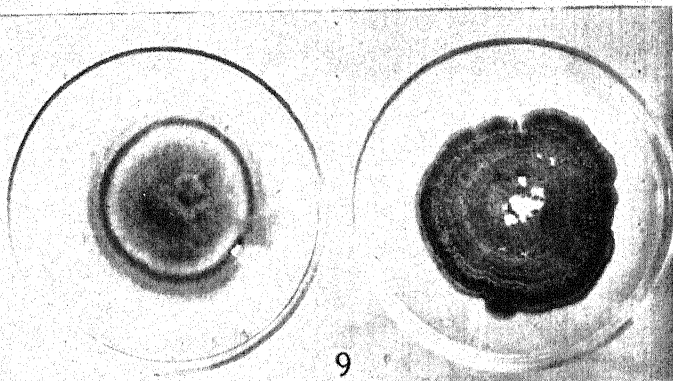
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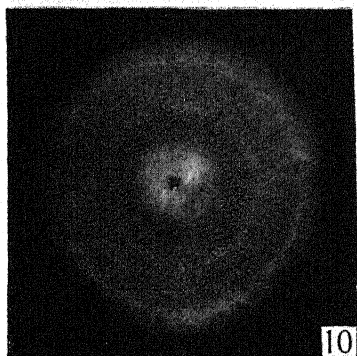
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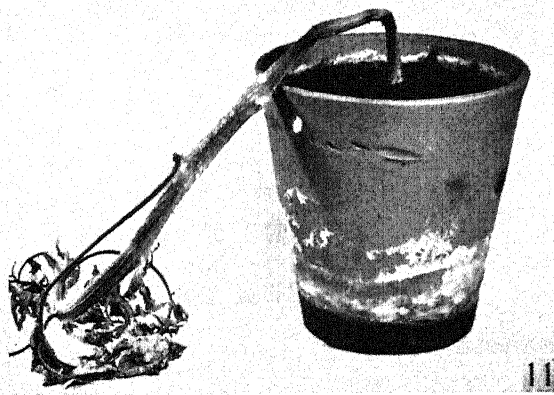
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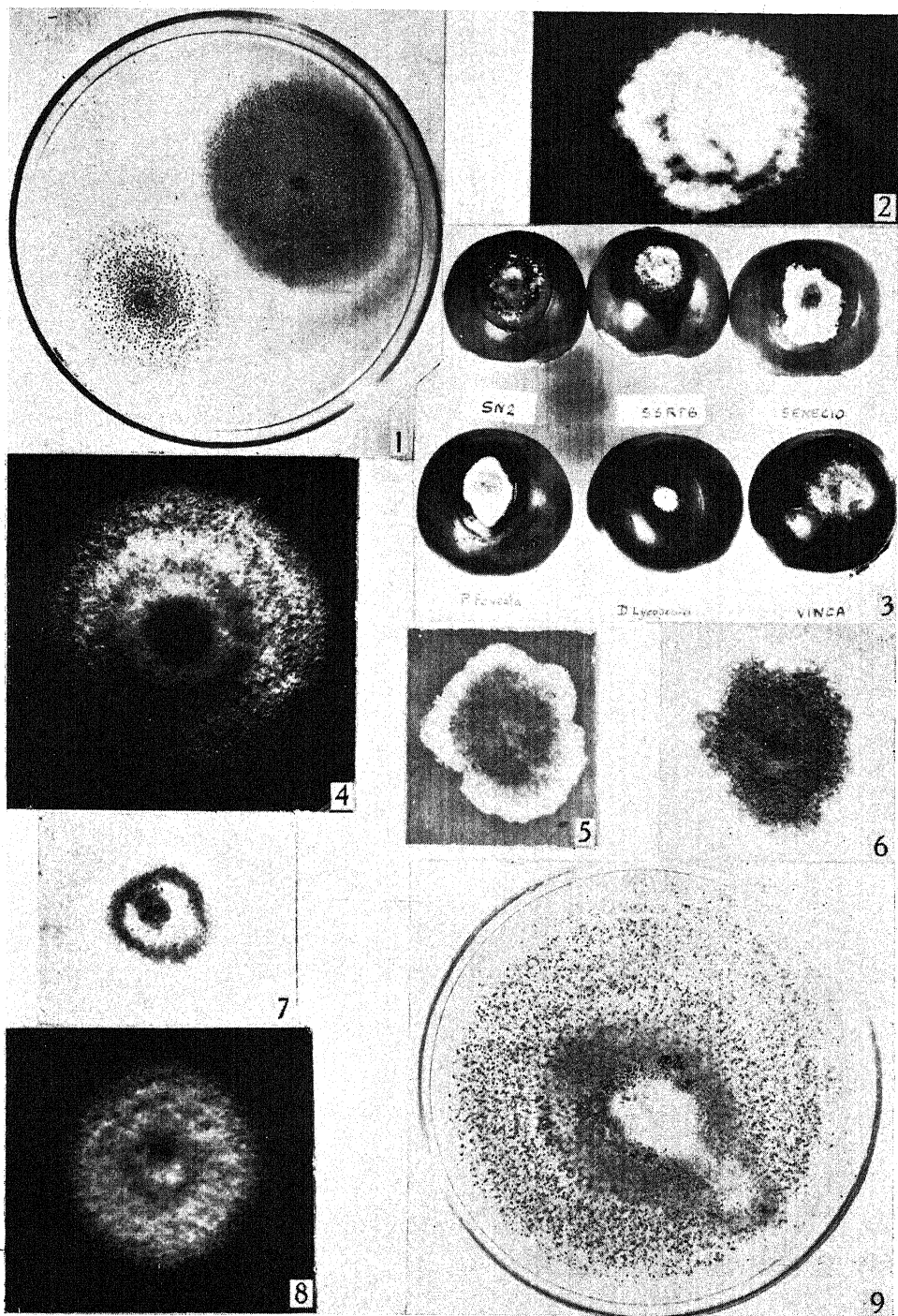
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EXPLANATION OF PLATES

All cultures photographed are on malt agar

PLATE I

- Fig. 1. Culture of strain 1, 6 days old.
Fig. 2. Lower surface of a 12 days old culture of strain 1 showing zoning.
Fig. 3. Culture of strain 31, 15 days old.
Fig. 4. Top left, strain 2; top right, strain 3; bottom, strain 4 after 16 days' growth on the same plate.
Fig. 5. Lower surface of a 12 days old culture of strain 2.
Fig. 6. Culture of strain 6, 9 days old.

PLATE II

- Fig. 1. Culture of strain 17, six days old.
Fig. 2. Culture of strain 19, 8 days old.
Fig. 3. Culture of strain 25, 8 days old.
Figs. 4-7. Cultures of strains 20, 21, 23 and 24 each 8 days old.
Fig. 8. Culture of strain 14, 8 days old.
Fig. 9. Cultures of strains 22 (left) and 23 (right), 1 month old.
Fig. 10. Culture of strain 26, 8 days old.
Fig. 11. Tomato plant killed by stem rot 6 weeks after inoculation with strain 7.

PLATE III

- Fig. 1. Strains 25 (left) and 27 (right) after 10 days' growth on the same plate.
Fig. 2. Strain 28, 8 days old.
Fig. 3. Tomato fruit rotted by strains 2, 3, 4, 1, 11 and 16, 13 days after inoculation.
Fig. 4. Strain 30, 8 days old.
Fig. 5. Strain 7, 8 days old.
Fig. 6. Strain 10, 14 days old.
Fig. 7. Strain 32, 14 days old.
Fig. 8. Strain 29, 8 days old.
Fig. 9. Strain 34, 14 days old.

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SPORE DISCHARGE IN *DALDINIA CONCENTRICA*By C. T. INGOLD, *Birkbeck College, University of London*

(With 7 Text-figures)

Amongst angiosperms physiologists recognize a number of kinds of xerophyte. Two of the most outstanding are: (1) the succulent type (e.g. *Cactus* spp.) in which the plant body is, in the main, a reservoir of water, and (2) the drought-enduring type such as the creosote bush (*Covillea glutinosa*) in which growth ceases during the dry months and the small leaves pass the summer in a wilting condition reviving with the autumn rains (Maximov, 1929, p. 258).

Buller (1909 and 1922) has pointed out that the leathery and gelatinous lignicolous members of the Hymenomycetes are xerophytes of the drought-enduring type, and most lignicolous Pyrenomycetes are probably of the same physiological type. In most of the stromatal Pyrenomycetes growing on wood, spores are probably discharged from the perithecia only during wet periods when the fungal tissue is fully turgid. This has, for example, been clearly demonstrated for *Endothia parasitica*, the cause of American Chestnut Blight, and Anderson (1913) came to the conclusion that 'the ascospores are ejected into the air from the ostioles during rain and as long as the bark remains wet'. Heald and Studhalter (1915), studying the same fungus, found also that expulsion of ascospores occurs only during and immediately after rain when the stromata are fully moist, and that from March to October discharge occurs during each spell of rain. Since violent spore discharge in Pyrenomycetes involves the bursting of fully turgid cells (the asci) sufficient water must clearly be available for the perithecia if discharge is to continue.

Daldinia concentrica Ces. & de Not., whose large black perithecial stromata are such conspicuous objects on dead trunks and branches of ash, is also a xerophyte, but of a different type. It would seem to be a succulent xerophyte, the stromatal tissue representing a large water reserve, with the result that spore discharge can continue during long periods of drought. This paper is concerned mainly with the evidence for this statement.

Daldinia concentrica is probably the largest of the stromatal Pyrenomycetes of the British flora. The stroma is roughly hemispherical and often has a volume of 50 c.c. or more. The only British pyrenomycete in which the stroma is developed to a comparable degree is *Xylaria polymorpha*, and it will be interesting to see if that species resembles *Daldinia* in the physiology of its spore discharge.

In *Daldinia concentrica* the spores are normally discharged violently to a distance of 1.0-1.2 cm. If a detached stroma is placed on a piece of white paper in the still air under a glass cover, a deposit of sooty spores accumulates as a black band extending around the fungus to a uniform distance of 1.0-1.2 cm. Under certain conditions spores fail to be discharged violently

and escape instead in the form of spore tendrils as described by Bayliss Elliott (1920), but this method of spore liberation is uncommon under natural conditions.

In studying the daily march of spore discharge the arrangement shown in Fig. 1 was used. A glass slide supported on two wooden blocks was arranged over a detached stroma leaving a space of 1–2 mm. between the upper surface of the fungus and the lower surface of the slide. The fungus was freely exposed to dry laboratory air and there was no external water supply on which it could draw. Spores discharged from the upper, more or less flat, part of the stroma collected on the glass slide and the maintenance of the slide in that position prevented the upper surface of the stroma from becoming smothered by discharged spores. At approximately three-hourly intervals the rate of spore discharge was determined. For this a slide was used with a square centimetre, subdivided into square millimetres, etched upon it. This was substituted for the slide *S* (Fig. 1) and, by

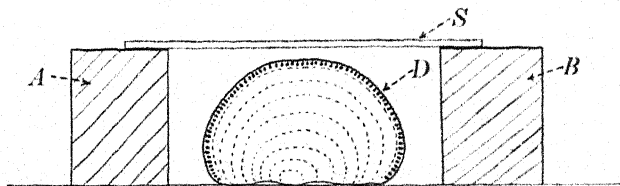


Fig. 1. Sectional view of arrangement for studying rate of spore discharge. *A* and *B*, wood blocks; *S*, glass slide; *D*, stroma of *Daldinia*.

fitting it to marks on the blocks *A* and *B*, the etched square was made to occupy, at each test, the same position relative to the fungus, thus ensuring that discharge from the same area of the stroma was sampled. It was found most convenient to expose the slide for fifteen seconds and after this exposure it was removed and the number of spores on the etched square counted under the microscope.

The results of this experiment are shown graphically in Fig. 2. It can be seen that discharge is essentially nocturnal and this has been confirmed in all the numerous specimens which have been kept under observation in the course of this work. In its nocturnal discharge *Daldinia concentrica* resembles *Hypoxyton fuscum* (Ingold, 1933).

The number of spores caught on a square centimetre of glass surface held just above the fungus gives roughly the spore output from the same area of the stroma. However, the particular stroma under observation had a perithecia-studded surface of 20 sq.cm. The maximum number of spores caught on 1 sq.cm. of the slide in fifteen seconds was 5400. For the whole stroma this gives an estimated rate of 432,000 spores per minute. It is interesting to note that this is of the same order of magnitude as the average output by a mushroom (*Psalliota campestris*). Buller (1909) found that the average rate of spore liberation from a fruit-body 8 cm. in diameter during the forty-eight hours of its spore-fall period was 670,000 per minute.

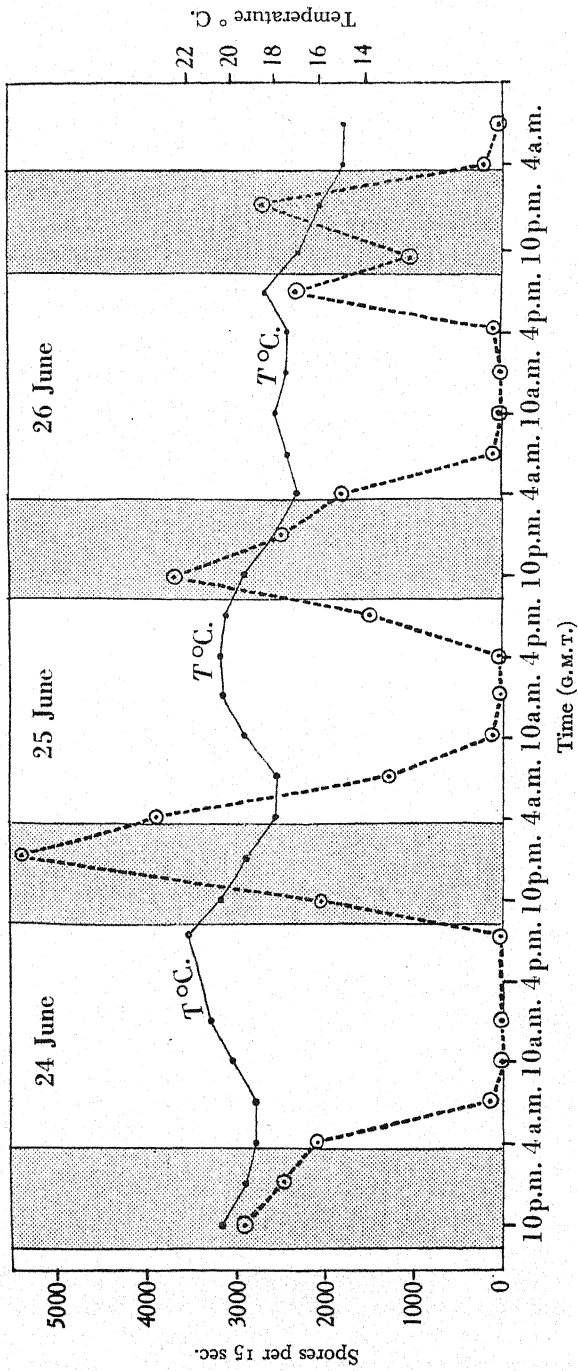


Fig. 2. Interrupted line: rate of spore discharge plotted against time. Continuous line: temperature. Periods from sunset to sunrise stippled.

It should be emphasized that the curve for spore-output plotted against time (Fig. 2) is based on few readings, since only eight observations were made each day out of a possible total of 5760 (i.e. the number of 15-second periods in a day). However, assuming the correctness of this graph and bearing in mind that the spores caught on the etched square represent only a twentieth of the total output of the stroma, an estimate can be made of the average daily spore production for the three 24-hour periods: 24-25 June, 25-26 June and 26-27 June. This works out at 133 million.

For the study of spore discharge from drying stromata the arrangement shown in Fig. 3 was used. A stroma was impaled on a blanket pin pushed

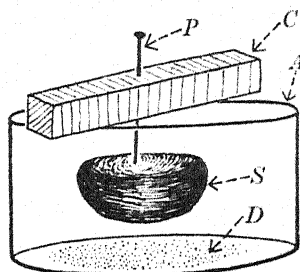


Fig. 3. Arrangement for collecting spore deposit. S, stroma; P, blanket pin; C, cork strip; A, crystallizing dish; D, spore deposit.

Table 1

	Original weight of stroma in g.	Original density	Period of spore discharge in days	Weight of stroma, when discharge ceased, in g.	Density when discharge ceased
A	4.83	—	20	1.35	—
B	26.02	0.96	27	6.10	0.25
C	34.48	1.01	33	6.92	0.21
D	24.69	1.02	26	6.84	0.31

through a strip of cork resting on the rim of a crystallizing dish, so that the discharged spores collected as a sooty deposit on the bottom of the dish.

In this series of experiments no quantitative determinations of spore output were made. Stromata of high density (approximately 1.0) were selected for study and each was exposed to dry laboratory air. It was noted daily if a visible spore deposit had formed overnight and the stroma was transferred to a clean crystallizing dish. In this way the spore discharge period for each specimen was determined, but it must be remembered that discharge may have been taking place for some time before the fungi were collected. During the course of the experiment each specimen was weighed at intervals. The results for four stromata are given in Table 1. Thus stroma C with an initial weight of 34.48 g. and a density of 1.01 continued to discharge spores for 33 days and only during the last ten days of this period was there any noticeable falling off in the density of the nightly spore deposit. When discharge ceased, the weight was reduced to 6.92 g. and the density to 0.21. During this period the change in volume was slight:

34 c.c. at the beginning, 32 c.c. at the end. The maintenance of the volume in spite of the considerable loss of weight is due largely to the rigid outer crust of the stroma.

The fact that spore discharge can take place for many days from a detached stroma has been recorded by Möller (1901), who, however, kept his specimens under a glass cover so that evaporation may have been slight. It must have been observed by many mycologists who have collected *Daldinia* and left stromata lying about in the laboratory. The surprising fact is that in dry air spore discharge can continue for so long.

In another series of experiments an attempt was made to give a quantitative estimate of the nightly output of spores during the drying of the stroma. The method used depends on the fact that if the spore deposit is suspended in a standard volume of water and if a definite depth of this suspension is interposed between a constant source of light and a photoelectric cell (Fig. 3), the suspension acts as a screen and the amount of light cut off due to the presence of the spores in the water depends on their concentration in the suspension.

Using a particular suspension, a series of dilutions was prepared (half-strength, quarter-strength and so on) and the amount of light absorbed by the original suspension and by each of the standard dilutions was determined, the zero value being given by water without any spores. From the data thus obtained a curve was constructed relating spore concentration, in arbitrary units, with the amount of light cut off by the suspended spores. This served as a calibration curve (Fig. 5).

In practice the nightly spore output was suspended uniformly in 50 c.c. of water and 20 c.c. of this suspension were poured into a glass vessel (*G* in Fig. 4) which was then placed immediately above the sensitive disk of a photoelectric cell. The reading given in foot-candles was subtracted from a reading obtained immediately before for which 20 c.c. of water were used in place of the suspension. From this value the spore concentration, in arbitrary units, was obtained from the calibration curve. For example, a particular suspension gave a reduction in light of 11.4 f.c. and this corresponded to a spore concentration of 5.4 *K*. *K* is a constant which can be estimated roughly. Using the same suspension twelve readings with a Thoma haemocytometer gave the spore-content of the suspension as 66.95 (s.e. 5.95) million. Equating this to the arbitrary value obtained by the photoelectric method gave a value for *K* of 12.4 (s.e. 1.1) million. Although the standard error (s.e.) is relatively high, this gives a clear picture of the order of magnitude of *K*.

The method of estimating spore concentration by using a photoelectric

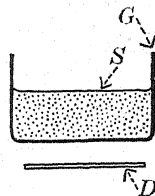
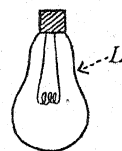


Fig. 4. Photoelectric method for measuring spore concentration. *L*, lamp; *G*, glass dish; *S*, spore suspension; *D*, sensitive disk of photoelectric cell.

cell is very sensitive and is also easy, a single determination occupying only a few minutes.

For the quantitative experiments the arrangement shown in Fig. 3 was placed in a desiccator containing anhydrous calcium chloride. In this way the air was kept not only dry but also still, so that practically all the discharged spores collected at the bottom of the crystallizing dish which was changed daily. Each nightly deposit of spores was suspended in water and the concentration determined by the photoelectric method. Two stromata were studied. Each had a density greater than 1.0 at the beginning of the experiment and each was weighed at intervals throughout. The results are recorded graphically in Fig. 6.

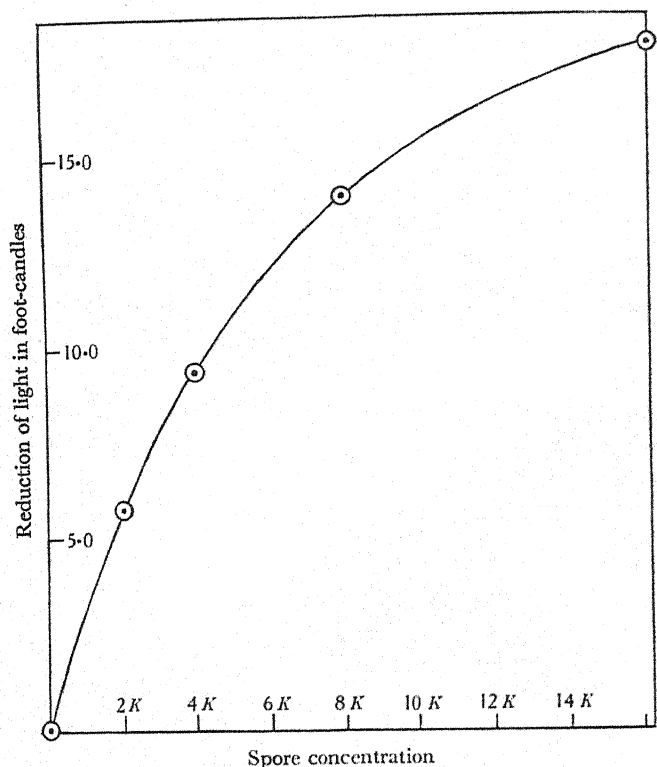


Fig. 5. Calibration curve relating reduction of light in foot-candles with spore concentration in arbitrary units.

It may be seen that for the first eleven days in the desiccator the rate of discharge showed no steady tendency to fall off. It fluctuated to some extent in both stromata probably due to variations in temperature, and it is interesting to note that during this period (1-11 June) the curves for the two stromata showed a clear parallelism with maxima on the three nights 2-3 June, 6-7 June and 10-11 June. After the first eleven days the rate of

discharge began to decrease steadily and finally, after 18 days for stroma *E* and 14 days for stroma *F*, discharge ceased. This final steady fall in spore output set in for *E* and *F* only when the weight of each had fallen to approximately one-half and discharge ceased when the weight was reduced to less than a third of the original.

The average daily spore output for the first eleven days in the desiccator was 155 million for *E*, and 69 million for *F*. These values compare very

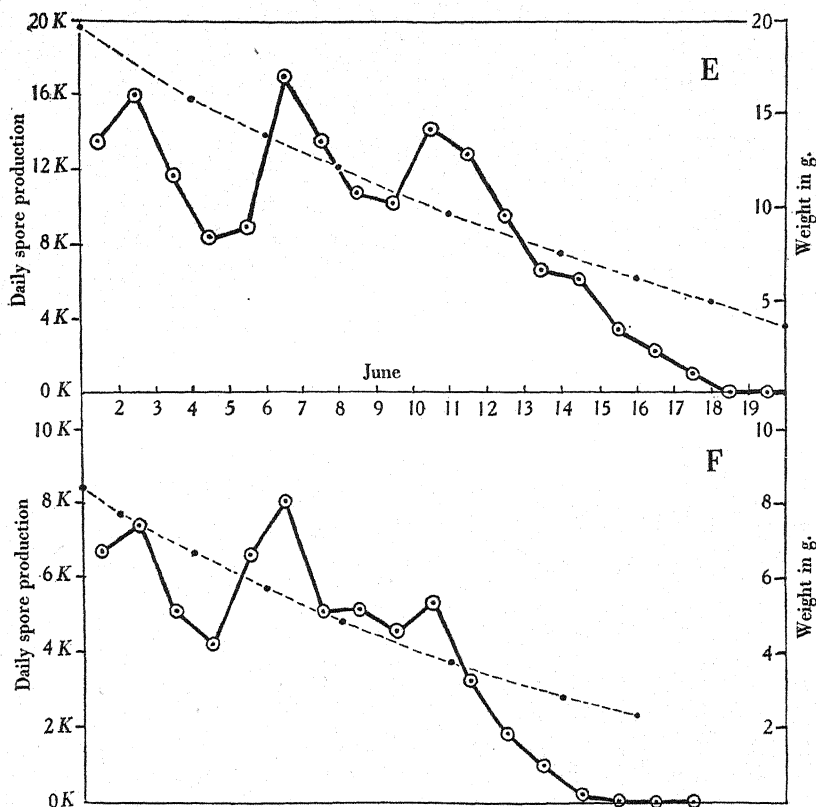


Fig. 6. Daily spore production plotted against time for two stromata (*E* and *F*) in a desiccator. Solid line shows daily spore production in arbitrary units. Interrupted line shows weight of stroma.

well with the figure of 133 million for the stroma used in the first experiment (see p. 44) which, however, had a volume about 50 % greater than that of *E*.

It is interesting to calculate what part of the loss of weight of each stroma during the period of active spore discharge is represented by the weight of the spores themselves. Discharged spores were mounted in water and 100 were selected at random and measured. The average dimensions were $14.2 \times 6.8 \mu$. Assuming that the spore is ellipsoidal with a density of 1.0, the weight of a million spores works out as 0.000344 g. The total spore output

of the stroma *E* during the experiment was 166.3 *K* which is approximately equivalent to 2062 million spores. The weight of these would be roughly 0.71 g. The loss of weight of the stroma during the period of active spore discharge was 15 g. Similarly, for stroma *F* the total spore output was 797 million corresponding to a weight of 0.27 g. and the loss of weight during the discharge period was 5.6 g. Thus the loss of weight is accounted for only to a small extent by the spores themselves and is probably mainly due to evaporation (transpiration), although some water is also lost as ascus sap discharged with the spores.

From the observations given above it is clear that *Daldinia concentrica* is a xerophyte capable of long-continued spore discharge under very dry conditions. It is suggested that this is possible because of the water reserve of the stomatal tissue, although this does not exclude the possibility that this tissue also contains a reserve of food. It may seem strange to suggest that this fungus is a succulent xerophyte. It does not feel succulent because of its hard outer crust, but if a stroma is examined during the early part of its discharge period, when its density is 1.0 or more, the stomatal tissue within the crust is found to be very juicy.

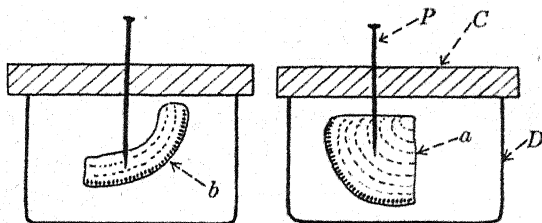


Fig. 7. Sectional view of arrangement used in studying spore output by half-stromata. *a*, half-stroma; *b*, half-stroma with some stomatal tissue removed; *P*, blanket pin; *C*, narrow cork strip not covering the dish *D*.

If the stomatal tissue behaves as a reserve, discharge of spores should cease earlier if part of this tissue is removed. To test this two similar experiments were carried out. In both experiments a fairly large and more or less hemispherical stroma was selected and cut into two half-hemispheres. One of these (*a*) was left without further treatment. The other (*b*) had part of its stomatal tissue scooped out, but a broad zone of this tissue was left below the perithecia to ensure, as far as possible, that they received no injury as a result of the treatment. All the cut surfaces in (*a*) and (*b*) were vaselined. The arrangement of the experiment is shown in sectional view in Fig. 7. It should be noted that the cork strip *C* did not cover the dish, so that the stomata were freely exposed to the dry laboratory air. The crystallizing dishes were changed daily and in both experiments the duration of spore discharge for the two halves (*a*) and (*b*) was determined. The results are shown in Table 2. In experiment *H* the weights of the two halves were originally: (*a*) 22.51 g. and (*b*) 23.09 g. and then from (*b*) 8.32 g. of stomatal tissue were removed.

Although it is unsafe to argue from experiments involving the mutilation of an organism, the results of these two experiments are, nevertheless, con-

sistent with the view that the stromatal tissue is a reserve, since with part of it removed spore discharge comes earlier to an end.

Within an active perithecium there does not appear to be any air, the asci and paraphyses being surrounded by a rather viscous fluid. When an active stroma is cut across, the liquid contents of the perithecia can easily be seen with a lens. It is only after spore discharge has ceased that a gas phase makes its appearance within the perithecia and, when this has happened, it does not seem possible to revive their activity even by placing the stroma with its base in water.

Table 2

	Duration of spore discharge in days	
	Half stroma (a)	Half stroma (b)
G	18	12
H	26	13

SUMMARY

The ascospores of *Daldinia concentrica* are normally discharged to a horizontal distance of 1.0–1.2 cm. Discharge is essentially nocturnal.

Spore discharge may continue from isolated stromata exposed to dry laboratory air for 20–30 days and during this time the density of the stroma decreases from about 1.0 to 0.2–0.3.

A method, involving the use of a photoelectric cell, is described for the rapid estimation of the nightly spore output in arbitrary units. Using this method, the nightly spore production of stromata enclosed in a desiccator was studied, and it was found that for the first eleven days spore output showed no tendency to decrease, although the density of the stromata fell from about 1.0 to about 0.5.

Experiments are described in which part of the stromatal tissue was removed and in consequence the period of spore discharge was considerably reduced.

It is suggested that *D. concentrica* is a xerophyte of the succulent type capable, by virtue of the water reserve in the stroma, of long-continued spore discharge under extremely dry conditions.

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ECOLOGY OF THE LARGER FUNGI

By JOHN GRAINGER, *Department of Plant Pathology,
West of Scotland Agricultural College, Auchincruive, by Ayr*

(With 6 Text-figures)

The British Mycological Society is founded upon a great tradition of field work. My home county of Yorkshire has inherited a long line of fungus forays, one of which provided the initial conference of this more famous daughter Society. Field work has laid the foundations of systematic mycology, which is still advancing. The present generation, however, not finding such a basic necessity for naming and classification, could begin the field investigation of ecology. Plant pathology, or applied mycological ecology, must always begin as a field study. It is invariably the better for outdoor inspiration.

Fungi are among the most prolific of living organisms. *Lycoperdon giganteum* probably holds the record, with a production of about 7,000,000,000,000 spores per fructification, and other species have very large numbers. Most of these spores die, but what is the mechanism of establishment of the infinitesimally small number which are successful? They must, among other factors, find a medium of suitable moisture content, with nutrient matter adjusted qualitatively and quantitatively to their needs, and of suitable relative acidity (pH). It is possible to measure some of the necessary conditions by the field methods of ecological survey. Of the three factors mentioned above, relative acidity is most easily measured in the field; estimations can be made on different substrates—soil, dung, and even wood and bark.

THE EFFECT OF pH OF THE SUBSTRATE

Methods

Measurement of pH of the soil can be carried out conveniently in the field by colorimetric methods. Most soils are within the range of British Drug Houses Soil Indicator (pH 4.0–8.0). Several permanent standards are available, but the most portable is the chart of printed colours prepared for the B.D.H. barium sulphate soil testing outfit. This, used with the ordinary soil testing kit, will give a reading with most soils and humus layers. Tests have been made of soil scraped from the bases of fructifications—perhaps the best arbitrary place for preliminary observations. Results are recorded on sheets 5 × 3 in., with all relevant data, and are filed as a card index. The very convenient refinement of specially printed sheets (Fig. 1) has been provided for the present investigations by Mr R. Fowler Jones, who has always helped Yorkshire mycologists in many ways.

Terrestrial species

Over 1400 estimations have now been made, and show certain possibilities of correlation. It had been expected that species would show a certain relation with *pH* of the substrate (Figs. 3 and 4 and Table 1), but it also appears that whole genera may favour certain sections of the *pH* scale (Figs. 2, 3). Clearly defined generic preferences for a particular limited range of acidity, such as are shown by *Russula*, *Lactarius* and *Coprinus*, seem to reinforce the soundness of these systematic groupings, apart from other considerations. Wide toleration of relative acidity, as in the genus *Hygrophorus* might be taken as an indication of a group still undergoing change.

Name Date

Loc.

Associated Plants

Coll. Det. Kind of Spore

Host Alt. Host.

Soil	<i>pH</i>	% Water	% Org. Matter				

Fig. 1. Sheet for recording ecological data. The headings 'Kind of Spore', 'Host' and 'Alt. Host' are used for studies on the ecology of Rusts and other fungi not considered in this paper.

Table 1 shows the *pH* range favoured by several of the more common species for which most records are available. Most species have a preference for moderately acid conditions, though a minority favour neutral or slightly alkaline reaction.

James Bolton, in his volume *History of Fungusses growing about Halifax* (1788), mentions *Morchella esculenta* as growing round that town. It cannot be found there now and, with increasing industrial development of the district, a considerable 'limestone' flora of higher plants has also disappeared (Grainger, 1942). The present investigation shows that *Morchella* requires soil of *pH* 7.0-8.0. It would seem that the ground, once sufficiently alkaline in parts for the growth of *Morchella* and several lime-loving plants, has gradually become more acid, and this has eliminated the calcicolous species.

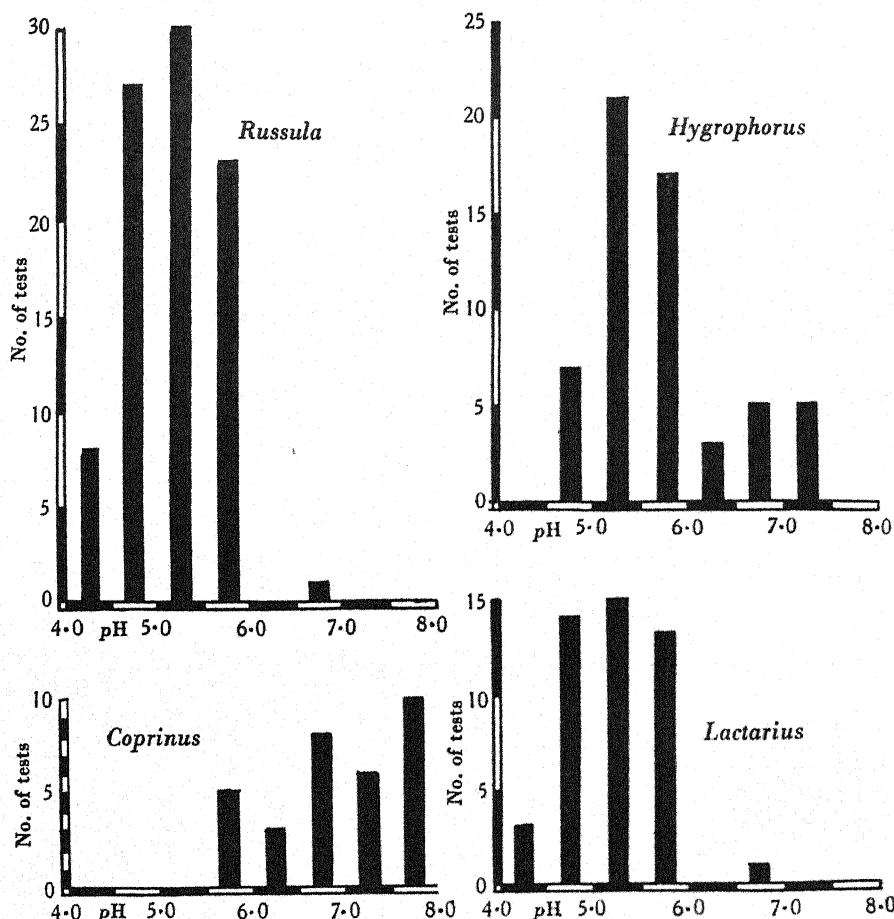


Fig. 2. pH requirements of fungal genera. The estimations include the following numbers for each species:

RUSSULA, 88 determinations: *adusta* 2, *atropurpurea* 17, *cutefracta* 1, *cyanoxantha* 14, *elephantina* 1, *enetica* 13, *fragilis* 1, *graminicola* 1, *lactea* 2, *luteotacta* 1, *nigricans* 10, *ochroleuca* 19, *olivacea* 1, *Velenovskyi* 4, *venosa* 1.

HYGROPHORUS, 57 determinations: *calyptraeformis* 5, *ceraceus* 5, *chlorophanus* 10, *coccineus* 5, *conicus* 7, *eburneus* 1, *metapodius* 2, *miniatus* 1, *nigrescens* 1, *niveus* 1, *pratensis* 6, *psittacinus* 8, *puniceus* 2, *subradiatus* 1, *virginicus* 2.

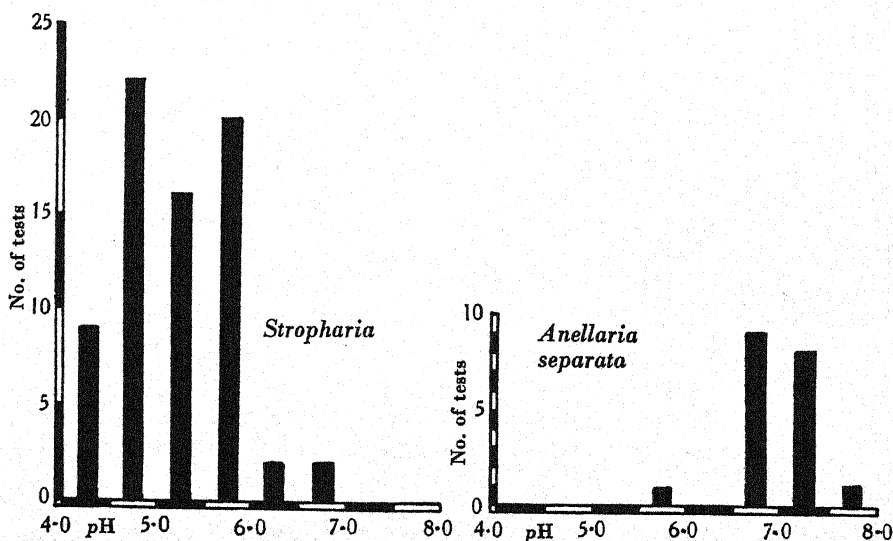
COPRINUS, 32 determinations: *atramentarius* 3, *comatus* 4, *lagopus* 2, *micaceus* 17, *niveus* 1, *papillatus* 1, *plicatilis* 3, *radiatus* 1.

LACTARIUS, 46 determinations: *camphoratus* 1, *ichoratus* 2, *piperatus* 2, *pyrogalus* 4, *quietus* 3, *rufus* 3, *subdulcis* 16, *subumbonatus* 6, *subtomentosus* 1, *torminosus* 1, *turpis* 7.

The common mushroom, *Psalliota campestris*, is found more often on substrata between pH 6.5 and 7.5, and has not yet been observed below pH 5.8 (Fig. 4). This fact should have significance for mushroom growers, for the erratic behaviour of this fungus in cultivation must owe a good deal to inappropriate reaction of the manure and casing soil. One bed which failed to produce mushrooms was partly distributed over a border prepared

Table 1. pH ranges of some common fungi

	pH range	No. of determinations
<i>Amanita rubescens</i>	4.0-5.8	21
<i>Amanitopsis fulva</i>	4.5-5.8	10
<i>Anellaria separata</i>	5.8-8.0	16
<i>Boletus chrysenteron</i>	4.7-6.7	11
<i>B. edulis</i>	4.5-6.0	14
<i>Collybia maculata</i>	3.5-5.5	13
<i>Coprinus micaceus</i>	5.8-8.0	17
<i>Hygrophorus chlorophanus</i>	5.0-7.4	10
<i>Hypholoma fasciculare</i>	4.0-6.8	17
<i>Laccaria laccata</i>	4.8-6.3	25
<i>Lactarius subdulcis</i>	4.0-5.8	16
<i>Lycoperdon pyriforme</i>	5.7-6.7	12
<i>Morchella esculenta</i>	7.0-8.0	7
<i>Panaeolus phalaenarum</i>	5.7-7.2	15
<i>Paxillus involutus</i>	4.0-5.8	27
<i>Phallus impudicus</i>	3.5-7.0	18
<i>Psilocybe semilanceata</i>	5.0-6.8	16
<i>Russula atropurpurea</i>	4.0-5.8	17
<i>R. cyanoxantha</i>	4.2-5.9	14
<i>R. emetica</i>	4.8-6.0	13
<i>R. nigricans</i>	5.2-6.0	10
<i>R. ochroleuca</i>	4.0-5.8	19
<i>Scleroderma aurantium</i>	4.0-5.8	17
<i>Stropharia semiglobata</i>	4.0-6.0	55

Fig. 3. pH requirements of coprophilous fungi. Estimations for the genus *Stropharia* include the following number for each species: *semiglobata* 56, *merdaria* 8, *stercoraria* 7.

for tomatoes, on which the fungi then appeared. Investigation showed that the original manure was pH 4.8 and the casing soil pH 5.0, showing that conditions were too acid for the mushroom. The tomato compost, from which the mushrooms grew, was pH 6.0, i.e. within the tolerance of *Psalliota campestris*. Many growers add lime and gypsum to their mushroom composts, and this should always be done, in order to attain a pH of 6.5–7.0.

Coprophilous species

Fungi which grow on dung also show marked preference for particular ranges of relative acidity. *Stropharia semiglobata* favours more acid dung, whilst *Anellaria separata* tends towards alkaline conditions (Fig. 3). The fullest test of this idea was to find both species on the same piece of dung, but each fungus was growing in its appointed sphere of pH: it was the dung which varied. Variation could result from the kind of food given to the voiding animal, and the length of time after voiding. Alkaline specimens are usually those more freshly deposited.

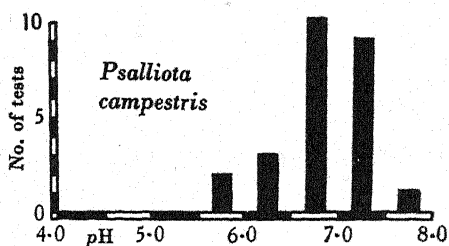


Fig. 4. pH requirements of *Psalliota campestris*.

Humaria granulata is often the first species to appear on alkaline cow manure, and *Anellaria separata* may follow. *Stropharia semiglobata* and *S. stercorearia* are not particularly prevalent until the later stages of decay, when the dung patch is becoming somewhat broken and decrepit. There is thus an ecological indication that dung, though it may be voided at varying pH, nevertheless undergoes a progressive change towards greater acidity as it lies upon the surface. Leaching by rain may also play a part in this change.

Lignicolous species

It is not easy to determine the relative acidity of wood. Colorimetric estimations with thymol-blue and cresol-red indicators would place it about pH 2.9, but this may not represent the effective reaction of the whole tissue. It seems fairly certain, however, that fungi which grow upon fresh or living wood, have chosen one of the most acid substrates in nature. Fallen logs, as they decay, however, undergo a progressive change of pH towards neutrality. There is also a change in texture or 'hardness' which can be measured by estimating the pressure required to drive a metal point into the wood, up to one or more arbitrary marks. An apparatus similar to that shown in Fig. 5 was used for this purpose. Fresh soft woods require a

pressure of 30–40 lb., and hardwoods a pressure of 40–50 lb. for the point to penetrate to the second line. When the wood is rotten, the point will penetrate to the full depth with only 3 or 4 lb. pressure. The instrument reinforces pH measurements in assigning intermediate points of decay, though their readings are not by any means parallel. *Xylaria hypoxylon*, for example, seems to favour wood which is approaching neutrality (pH 7.0) but which still retains about 80 % of the hardness of the fresh substrate. *Fomes annosus*, on the other hand, appears to be able to grow on wood which retains only 20–25 % of its original hardness, but is still fairly acid—pH 5.0. It would seem that two different types of decay are represented by these examples. Succession of fungi upon fallen logs doubtless owes much to the

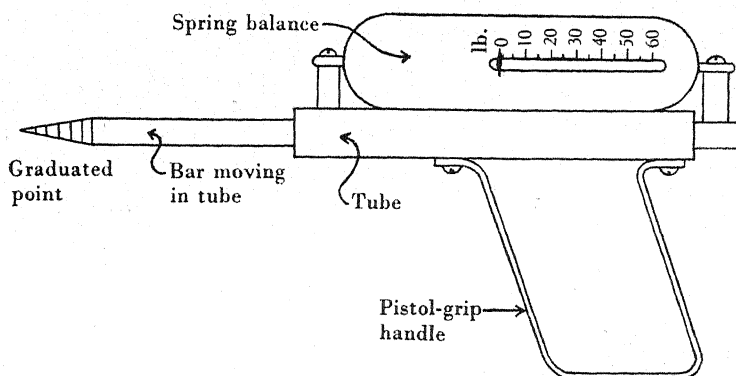


Fig. 5. Diagram of an apparatus for measuring 'hardness' or stage of decay in wood.

stage of decay presented to successive depositions of spores, but the fungi also contribute to the decay. *Trametes mollis* reduced the hardness of wood to about 27 % of its initial value, and *Volvaria volvacea* grew on wood which had a hardness 25–32 % of that on which the fungus was not growing. There are many difficulties in this work, and Table 2 is included, but tentatively, to show potentialities of this line of ecological approach.

Table 2. Ecology of succession of fungi upon fallen logs

	pH	'Hardness' of habitat (lb.)	'Hardness' of healthy wood (lb.)	% reduction in 'hardness'
<i>Stereum rugosum</i>	4.3	12–16	30–40	60
<i>Pluteus cervinus</i>	5.5	15	42	64
<i>Fomes annosus</i>	5.0	9	45	80
<i>Xylaria hypoxylon</i>	7.0	32	40	20

Bark is throughout much more nearly neutral than wood. Colorimetric readings have varied between pH 6.0 and 7.3, but here again these values may not represent the reaction of the whole tissue. The ecologist must be careful to distinguish between actual growth of a fungus on the bark, and mere fructification thereon. *Stereum purpureum*, for example, can certainly grow on wood, but fructifies on the bark, whereas *Nectria cinnabarina* can probably draw the greater part of its nutrition from the bark.

REQUIREMENTS OF WATER AND ORGANIC MATTER

Methods

It is necessary to weigh in the field samples of soil or loam destined for moisture determinations. This usually needs shelter for the hand balance which is used. Material can be filled into a tared crucible, reweighed, and then carefully wrapped in paper bearing a note of both weights, and other data for the sample. This can then be dried and incinerated as soon as convenient.

Table 3 suggests that, for growth and reproduction of the larger terrestrial basidiomycetes, water-holding capacity of the substratum is probably of

Table 3. *Requirements of water and organic matter*

	Average water content % of fresh wt.	Average organic content % of fresh wt.	No. of determinations
<i>Amanita rubescens</i>	38.5	12.4	11
<i>Galera hypnorum</i>	53.5	15.6	5
<i>Hygrophorus chlorophanus</i>	42.9	11.5	5
<i>Laccaria laccata</i>	40.2	14.4	13
<i>Lactarius pyrogalus</i>	27.6	6.9	4
<i>L. subdulcis</i>	56.6	21.2	5
<i>Lycoperdon pyriforme</i>	41.6	10.1	5
<i>Paxillus involutus</i>	46.5	24.2	15
<i>Phallus impudicus</i>	56.6	25.3	5
<i>Psilocybe semilanceata</i>	46.6	14.7	5
<i>P. coprophila</i>	61.7	22.5	4
<i>Russula atropurpurea</i>	46.9	24.3	7
<i>R. cyanoxantha</i>	39.6	15.7	7
<i>R. emetica</i>	59.3	26.7	10
<i>R. ochroleuca</i>	47.5	22.6	11
<i>Scleroderma aurantium</i>	46.1	15.6	7
<i>Stropharia semiglobata</i>	63.6	23.7	18

more importance than the amount of organic matter *per se*. The average water contents in the table are those of the habitat, but several determinations suggest that they are usually close to the water-holding capacities of the composts at the time fungal fructifications appear. Perhaps the most significant fact is that the water contents are very much higher than those of arable land, where moisture determinations over 30% are rare. This would account for the disappearance of the larger fungi when grassland is ploughed; the surface layer of organic matter, which is mainly responsible for holding the larger quantities of water, is broken and dispersed in the soil. Some basidiomycetes can, however, grow and fructify on the new conditions; *Lactarius pyrogalus* of Table 3 would probably succeed, and at Meltham, *Russula nigricans* was the sole species which survived the ploughing of an old pasture with a rich and varied fungus flora, as detailed below. The low amount of organic matter required by *Lactarius pyrogalus*, and the high water contents associated with the two coprophilous species *Stropharia semiglobata* and *Psilocybe coprophila* are other noticeable features of Table 3.

CHANGES IN THE FUNGUS FLORA

Do fungi appear in the same station in successive years? Are there annual and perennial mycelial masses? How far is the fungal flora of a given area liable to change from year to year? To what extent is the fungus flora dependent upon the ecology of higher plants? These questions, which animated James Bolton in 1788 (see also Grainger, 1940), cannot yet be answered very fully. A certain amount of field work, however, can provide suggestions and indicate the relative importance of the ecological factors mentioned in previous sections.

Habitat and succession

Two half-acre plots have been kept under detailed observation by Miss J. Grainger for the last four years, every fungal fructification being counted at approximately weekly intervals. By great good fortune, both habitats were changed during the counts.

The plot at Reservoir field, Meltham Mills, near Huddersfield, was a

Table 4. *Total number of sporophores, Reservoir field—old pasture*

	Average pH 5.0			Average pH 6.0
	1942	1943	1944	1945
<i>Amanita rubescens</i>	66	32	50	—
<i>Boletus badius</i> *	12	6	15	—
<i>B. edulis</i> *	13	—	—	—
<i>B. erythropus</i>	5	5	—	—
<i>B. subtomentosus</i>	—	1	17	—
<i>Collybia butyracea</i> *	—	1	23	—
<i>Hygrophorus laetus</i>	—	29	123	—
<i>Russula xerampelina</i>	13	1	3	—
<i>R. fellea</i>	32	6	42	—
<i>R. emetica</i> *	2	14	21	—
<i>R. nigricans</i> *	1	9	—	3
				↑ Ploughed, limed and reseeded

* Species whose tolerance of pH 6.0 has been established by previous survey.

very old pasture, running back almost to rough moorland, and dominated by *Aira flexuosa*. It was ploughed in the spring of 1945, limed and reseeded.

Table 4 shows that the fungus flora varied considerably in the three years before ploughing, but after that disturbance the change was drastic. *Russula nigricans* was the sole survivor. Liming raised the pH of the ploughed soil from an average of 5.0 to an average of 6.0. Five species shown by an asterisk (*) in Table 4, are known, from the pH survey discussed earlier in this paper, to be able to grow and fructify at pH 6.0. *Amanita rubescens* could still be found on an unploughed headland of the old pasture, to which lime had been added (pH 5.8). It does not seem that change in soil reaction alone could account for the drastic change following ploughing, liming and reseeded. Change of water-holding capacity by ploughing has already been discussed, and this is deemed to be the major factor. In support of this idea, the moisture-holding capacities of ploughed and

unploughed soil, both of which had been limed, were found to be 58.5 and 31.4 % respectively. From Table 3, it would seem that the ploughed soil could not hold sufficient water for the growth of most fungi; *Russula nigricans* is an exception, which has frequently been found on very dry situations.

The second plot was in Banks Wood, close to the first. It was a mixed oak and beech wood, with ground vegetation of *Holcus* and *Aira*. It was felled in the winter 1942-3.

Table 5 shows that *Boletus chrysenteron* disappeared with the trees; the other three species diminished more slowly. There was no significant change in pH, and in 1945, it was pH 4.5-5.0, within the limits already established for the four species of Table 5. *Amanitopsis vaginata* and *Boletus chrysenteron* often grow right away from woods, so it is unlikely that disappearance of the trees removed any element directly essential for their

Table 5. Total number of sporophores, Banks Wood (mixed oak)

	1942	1943	1944
<i>Amanitopsis vaginata</i>	36	16	—
<i>Boletus chrysenteron</i>	100	—	—
<i>Russula adusta</i>	33	1	—
<i>Scleroderma aurantium</i>	15	14	4

↑
Wood felled

immediate nutrition. The removal of trees would, however, probably disturb the water relations of the soil below, for evaporation would be greater after the trees had gone. Lack of sufficient water would seem to be the important factor in this case also.

Annual and perennial mycelium

Fairy rings of the terrestrial species *Entoloma porphyrophaeum* and *Lactarius turpis* appeared in fields at Meltham in 1943, but not a vestige of either could be found in 1944 or 1945. Several hundred fructifications of *Armillaria mellea* arose from the ground away from trees in 1943. The species had not fructified there for three years previously, nor did it appear in 1944 and 1945. Changing frequency of humicolous species from year to year would doubtless reflect the great element of chance involved in spore distribution. It would also seem to fit the assumption that their mycelial masses are usually annual. On the other hand, the lignicolous species *Stereum karstenii* Bres. has only been found in Yorkshire at Buckden, in 1922, when it was the first British record, and again in 1936. *Panus torulosus* fruited on the same log, and at the same place on the log, two years in succession at Cawthorne in 1941 and 1942. *Polyporus betulinus* can fruit on the same birch tree for at least two successive years. Continuity of the individual in lignicolous species over more than one year would seem to indicate a possibility of perennial mycelium. The well-known annual layers of *Fomes annosus* provide further direct evidence of perennial growth.

PERIODICITY AND CLIMATE

The concentration of fructification of the agarics into an autumn maximum raises an interesting question in ecology. *Psalliota campestris*, when provided horticulturally with suitable climate and nutrition, will grow and fructify at any time of the year. The larger basidiomycetes, when grown in artificial

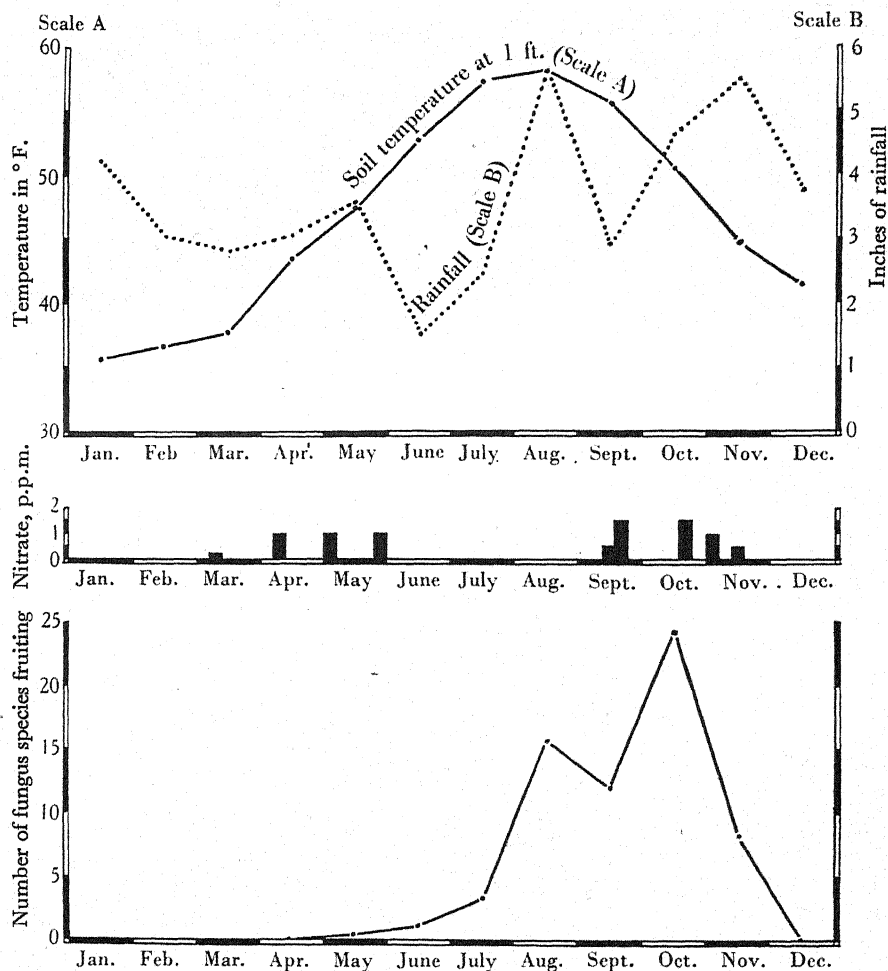


Fig. 6. Meteorological data, nitrate values and periodicity of fungus fructifications, Meltham Mills, Huddersfield; averages for 1941, 1942 and 1944.

culture, can fruit in any month. Natural conditions impose an autumn maximum, for which environmental factors might account. Mrs Mary Grainger (1942) has suggested that autumn is the only time when there are, simultaneously, a sufficiently high soil temperature, with enough rainfall for high soil moisture, and adequate supplies of available nitrogen. Spring

provides available nitrogen and soil moisture, but has low soil temperature. Summer brings a high enough soil temperature, but soil water is low and spare nitrogen is at a minimum. Winter has little nitrogen, low soil temperature, and perhaps more than adequate soil moisture. Such is the working hypothesis.

Weekly records of fungal fructifications made over four years in one locality by Miss Jennie Grainger, have been combined with meteorological data obtained by Mr A. Broadbent at a station about one mile distant. Nitrate measurements were also made on water draining from the site, using the presumptive method of Thresh, Beale and Suckling (1933). This was taken as a rough indication of nitrogen level in the soil. The collective results are shown in Fig. 6 and support the hypothesis. Each factor has *some* effect. Low rainfall in September, for example, is correlated with fewer species of fungi in fruit. No single factor, or combination of two factors, however, is adequate to explain the observed fungal periodicity. Maximum fructification in October is correlated with the only month when nitrogen was abundant, soil temperature over 50° , and rainfall over four inches. All other months fall short of this triple desideratum.

SUMMARY

Species of the larger fungi have definable preferences for particular ranges of pH of the substrate. The majority favour acid conditions but a minority prefer alkaline reaction.

Some genera also appear to have similar definable preferences.

The ecology of coprophilous species depends very largely upon reaction of the medium.

Most humicolous basidiomycetes require a high average water content in the substrate. Studies of two ecological changes suggest that this factor is of major importance.

The autumnal maximum of fungal fructification reflects the only time of year when soil temperature, soil water, and the level of available nitrogen, are simultaneously sufficient.

Ecology of the lignicolous fungi can be investigated in the field by correlated measurements of pH and 'hardness' in order to determine the particular type and stage of decay. Rotting wood generally undergoes a change from very acid to neutral reaction; bark provides approximately neutral conditions throughout.

This survey is designed to be provocative rather than finite. It is the result of using simple field methods, but indicates many possibilities of ecological investigation. It has had all the social value of a foray, being a co-operative work with Miss Jennie Grainger, Mrs Mary Grainger, Mr A. Broadbent, Mr C. Ridgwick, and members of the Mycological Committee of the Yorkshire Naturalists' Union. Mr A. A. Pearson kindly helped with the determination of several species.

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UROMYCES GENISTAE-TINCTORIAE (PERS.) WINT. IN SCOTLAND

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In September 1934, I found sori containing uredospores of *Uromyces Genistae-tinctoriae* on the leaves of *Genista anglica* near Inverness (Wilson, 1934). The *Genista* grows among heather at the side of Loch Duntelchaig about 700 ft. above sea-level. The rust was collected in the same locality yearly till 1941. Uredospores only have been found. The sori appear to be confined to the leaves, and are mostly hypophyllous. Leaves of the current season bear sori from June to October.

A large number of seeds was collected from infected plants in 1935. Plants raised from them in St Andrews all remained healthy.

Genista anglica and *Sarcothamnus* (*Cytisus*) *scoparius* are the only species included in the Sydows' (1904) list of host plants for *Uromyces Genistae-tinctoriae*, which occur at Loch Duntelchaig. The hosts listed include other species of *Genista* and several species of *Cytisus* and *Laburnum*. *Cytisus scoparius* and *Ulex europaeus* are both quite common near the loch. They have been examined frequently for signs of infection but with negative results.

Dr Malcolm Wilson has very kindly shown me his draft notes on *Uromyces Genistae-tinctoriae*, collected for his projected revision of Grove's (1913) *British Rust Fungi*. From these it is clear that the presence of the accidial stage of this rust in Britain has not been satisfactorily established. The accidium has been recorded on the continent on *Euphorbia Cyparissias* by Dietel (1919) and Kobel (1921), and on *E. virgata* by Treboux (1912). The former species is British and Druce (1932) gives its range from South Devon to Westernness. The nearest parts of this latter vice-county are over twenty miles distant from Loch Duntelchaig, and far inside its borders stretch mountain and moorland. It is therefore safe to assume that, in this area, *Uromyces Genistae-tinctoriae* lives as a repeating rust.

In September 1936, numerous plants of *Cytisus scoparius* and *Ulex europaeus* were examined on the banks of the Caledonian Canal at Inverness. One or two plants of *Cytisus scoparius* had their leaves infected with the uredospores of a rust. Microscopic examination showed that the spores were similar in appearance to those occurring on *Genista anglica*. The following measurements show that the spores on *Cytisus* tended to be more nearly equal in length and breadth and that the largest of them were slightly longer and markedly broader than the largest measured from *Genista*.

Genista anglica uredospores from leaves: $21.1-28.8 \times 18.6-22.5 \mu$; average $24.6 \times 20.7 \mu$.

Cytisus scoparius uredospores from leaves: brown-walled, 3-4 germ pores; $20.9-29.3 \times 18.9-28.1 \mu$; average $23.9 \times 22.1 \mu$.

Only a very few teleutospores were observed. They were almost round, with a well-marked apical germ pore.

Both types of spore agree with Kobel's (1921) description of spores from this host.

Each year one or two bushes of *C. scoparius* are found infected, at the same spot. Uredospores are present from June to January. All leaves appear to be free from infection for the rest of the year, but a few branch infections do occur so that the plants are never entirely clear of the rust.

In December 1936, one or two uredosori were found on the spines of *Ulex europaeus* growing intermixed with one of the infected *Cytisus scoparius* plants. No other infected *Ulex europaeus* has been found so far. The spores differed considerably in appearance from those occurring on *Cytisus scoparius* or *Genista anglica* as the following measurements show.

Ulex europaeus uredospores from spines: $21.7-38.4 \times 18.3-21.9 \mu$; average $28.6 \times 20.3 \mu$.

Cytisus Laburnum three hundred yards distant is not infected.

The solitary infection on *Ulex europaeus* is very interesting. The natural assumption is that spores from *Cytisus scoparius* brought it about and that the rust on *C. scoparius* has not adapted itself properly to the new host. The experience of continental workers has been that any particular group of aecidiospores from *Euphorbia* is not able to infect by any means all the recorded hosts for the uredospore and teleutospore stages, e.g. Guyot (1937) infected *Cytisus Laburnum* with aecidiospores from *Euphorbia Cyparissias* but was unable to infect *Cytisus scoparius* from the same source. The inability of uredospores formed on one leguminous host to infect another has also been recorded. Both these facts indicate the presence of specialized races within the species. Kobel (1921) divides the species into three forms which differ slightly from each other in teleutospore characters. The hosts for form A are *Cytisus* spp. including *C. Laburnum*, and *Genista* spp. B is also found on *Cytisus Laburnum* and on *Caragana* spp. C is recorded on *Caragana arborescens* only. The position of the rust on *Cytisus scoparius* is regarded as doubtful.

Infection experiments were undertaken in an attempt to discover the relationship existing among the rust populations occurring near Inverness.

(1) In August 1938, leaves from *Genista anglica* and *Cytisus scoparius*, bearing uredosori, were brought to St Andrews. They were placed separately on wire gauze trays suspended inside bell-jars. One plant of *Genista anglica* was then placed under each jar for a period of forty-eight hours. Conditions were kept moist by lining the jars with damp blotting paper and sealing the foot with moist cotton-wool. Plants of *Cytisus scoparius*, *Ulex europaeus*, *Cytisus Laburnum* and *Genista tinctoria* were then treated in the same way. Great care was taken to keep the two lots of infective material separate at all times. The two sets of plants were stored in cold frames about two hundred and fifty yards apart. All plants were examined at regular intervals for a month. Of those exposed to infection from *Genista* uredospores, only *G. anglica* became infected. Uredospores from *Cytisus scoparius* infected that plant only. Infection was heavy on *Genista* and rather lighter

than under natural conditions on *Cytisus*. Sori formed on both leaf surfaces. All infected leaves were removed in both experiments. Ten days later a second crop of spores appeared on the leaves of *Genista anglica*. No more were formed on *Cytisus scoparius*. There were no stem infections on any of the plants. While no sori formed on *Ulex europaeus* as a result of exposure to spores from *Genista* or *Cytisus* the plants exposed to spores from the latter did develop brown spots on both leaf and stem spines. The brown, dead tissue contained hyphae quite similar to those of the rust in living leaf tissues of *C. scoparius*, but it was not possible to identify these hyphae.

These experiments were repeated in 1939, 1940 and 1941 with similar results.

(2) Seed of *Euphorbia Cyparissias*, the alternative host occurring in Britain, was obtained in the spring of 1940. In the summer of the same year two of the plants raised from this seed were placed in pots beside plants of *Genista anglica* which were infected. Two others were placed with infected plants of *Cytisus scoparius* at the same time. No infection of the *Euphorbia* occurred that season or during 1941. In addition, during the spring of 1941 two euphorbias were planted out under infected *Cytisus scoparius* bushes at Inverness and two were planted among infected *Genista anglica* at Loch Duntelchaig. These four euphorbias were not attacked by the rust.

When an opportunity occurs again I intend to repeat my attempts to infect *Euphorbia Cyparissias*. If this can be done there is always the possibility that the distinct forms of *Uromyces Genistae-tinctoriae* which appear to exist on *Genista anglica*, *Cytisus scoparius* and *Ulex europaeus* may be induced to change their hosts. Additional species too may become infected. The inability of the rust to attack *Euphorbia Cyparissias* in the experiments so far undertaken is to be expected because no teleutospores have been found on *Genista anglica* and very few on *Cytisus scoparius*. Teleutospores are obviously not necessary for the survival of the rust. The fact that it repeats on *Genista* and *Cytisus* shows that the uredospores are able to survive the fairly severe conditions which they sometimes experience.

Uromyces Genistae-tinctoriae as it occurs in the Inverness area is made up of biologically distinct populations on *Genista anglica* and *Cytisus scoparius*. There are slight morphological differences between the uredospores. A third population is almost certainly established on *Ulex europaeus*. Uredospores differ markedly from those occurring on the other two hosts. As only one infection has been found it has not been possible to try to infect *Genista* or *Cytisus* from *Ulex*. Mutation leading to pathogenicity has been recorded by Newton and Johnson (1940) in *Puccinia graminis Triticici*. It seems likely that by this means the rust on *Cytisus scoparius* has come to produce uredospores capable of infecting *Ulex europaeus* and that this colonization is still going on at the present time.

When experiments on these rust populations are more complete it may be found advisable to distinguish three species. In the meantime it seems safe to say that there are both biological and morphological differences in the rust material occurring on the three different hosts. The three populations may be tentatively distinguished from each other as forms within the

Uromyces Genistae-tinctoriae (Pers.) Wint. *J. A. Macdonald* 67

species *Uromyces Genistae-tinctoriae* (Pers.) Wint. The following nomenclature is proposed:

Uromyces Genistae-tinctoriae (Pers.) Wint. f. *anglicae* f.n. Forma specialis ex *Genista anglica*; uredosporis rotundatis vel ellipticis, $21.1-28.8 \times 18.6-22.5 \mu$.

Uromyces Genistae-tinctoriae (Pers.) Wint. f. *scoparii* f.n. Forma specialis ex *Cytisus scoparius*; uredosporis rotundatis vel ovatis, $20.9-29.3 \times 18.9-28.1 \mu$.

Uromyces Genistae-tinctoriae (Pers.) Wint. f. *Ulicis* f.n. Forma specialis ex *Ulex europaeus*; uredosporis ellipticis vel oblongis, $21.7-38.4 \times 18.3-21.9 \mu$.

This work was interrupted in 1941 but is now being resumed.

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NOTE

CHYTRIDIUM LECYTHII (INGOLD) N.COMB.By E. K. GOLDIE SMITH, *Royal Holloway College*

(With 1 Text-figure)

In March 1943, when working in Dr C. T. Ingold's laboratory at University College, Leicester, I had the opportunity of studying living material of *Rhizophyidium Lecythii* Ingold, parasitizing the rhizopod *Lecythium hyalinum*, from the same locality in which the fungus was originally found. The chytrid was described by Ingold (1941), but he failed to observe the discharge of the zoospores. However, in the living material at my disposal I was able to establish that dehiscence of the sporangium is by a lid which may either hinge backwards (Fig. 1, *E-F*) or become completely detached (Fig. 1, *A*) and that the zoospore has a single posterior flagellum.

Since dehiscence is by a lid, it is necessary to transfer this species from the inoperculate genus *Rhizophyidium* to the operculate genus *Chytridium*, and the name becomes, therefore, *Chytridium Lecythii* (Ingold) n.comb.

REFERENCE

INGOLD, C. T. (1941). Studies in British Chytrids I. *Trans. Brit. myc. Soc.* xxv, 41-8.

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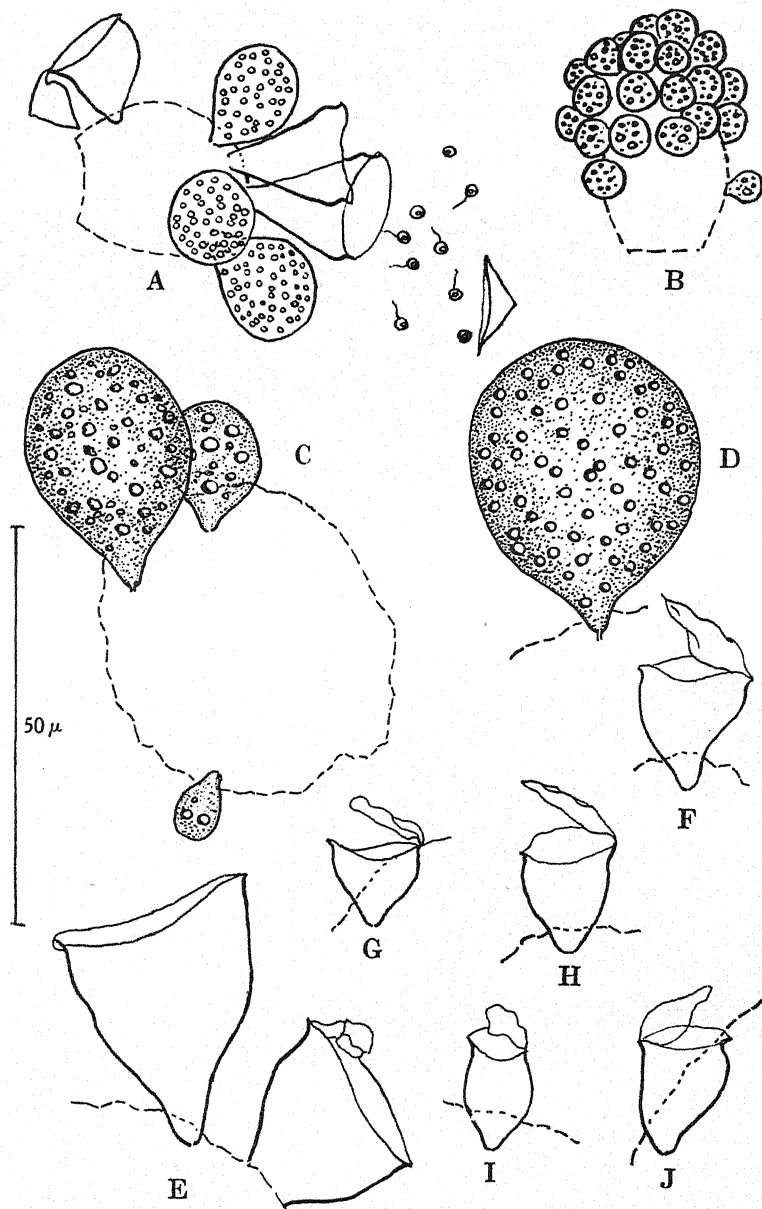


Fig. 1. *Chytridium Lecythii* on *Lecythium hyalinum*. A, B, Freehand sketches; C-J, camera lucida drawings. The surface of the rhizopod is indicated by a dotted line.

AN UNDESCRIBED SPECIES OF *CHAETOMIUM*, WITH FOUR-SPORED ASCI

By S. J. HUGHES

(With 1 Text-figure)

In November 1944, an unfamiliar *Chaetomium* arose as contaminant of an agar plate during an attempt to isolate a fungus parasitizing a wireworm; in pure culture the *Chaetomium* fruited abundantly on rabbit-dung agar with straw.

Through the kindness of the Director of Kew, and Miss E. M. Wakefield, material of all but four of the twenty-eight species of *Chaetomium* described by Chivers (1915) in his monograph have been examined. As far as I am aware, eight species of *Chaetomium* have been described as new just prior to or since 1915. These are: *C. fuscicolum* Petrak (1915), *C. subterraneum* Swift and Povah (Swift, 1929), *C. tortuosum* Garbowski (1936), *C. ochraceum* Tschudy (1937), *C. cancroideum* Tschudy (1937), *C. dolichotrichum* Ames, *C. microcephalum* Ames and *C. pachypodioides* Ames. Tschudy (1937) examined a culture of the type isolation of *C. subterraneum* Swift & Povah and found it identical with *C. globosum* Kunze.

Dr L. M. Ames very kindly sent me, on request, cultures of his own three species and also the two species erected by Tschudy in 1937.

In 1849 Fries described *Chaetomium hispidum* as follows: 'In *Ch. hispido* (gregario, atro, peritheciis hemisphaericis subnitidis, fibris sparsis rigidis divergentibus, in eodem caule jove pluvio monstrabat nucleum fluxilem, sporasque absque ascorum vestigio; sequente die sicco et sereno praebeuit ascos amplos, clavatos, pellucidos, sporis 4 seriatis ovatis simplicibus lutescentibus. Interdum deficit perithecium; gelatina floccis inspersa.'

Of this description Chivers states that 'No measurement of structures are given and it is impossible to arrive at a satisfactory conclusion regarding his material. The fact that the asci are four-spored would, in any case, exclude this species from *Chaetomium*.'

The *Chaetomium* described below differs from *C. hispidum* Fries in that it does not possess 'fibris sparsis rigidis divergentibus', 'ascos...clavatos' or 'sporis...ovatis...lutescentibus'. Singular characters of the terminal hairs weighed against the possibility of this fungus being a variety of one already described, assuming of course that, if a four-spored variety had arisen, the hairs would remain the same.

As the fungus differs markedly from the specimens of species examined and the descriptions of others it is proposed as new with the following diagnosis:

Chaetomium tetrasporum n.sp.

Perithecia grey, subglobose, ostiolate, 300–450 μ in diameter, on a poorly differentiated tuft of rhizoids. Lateral hairs numerous, of two sorts:

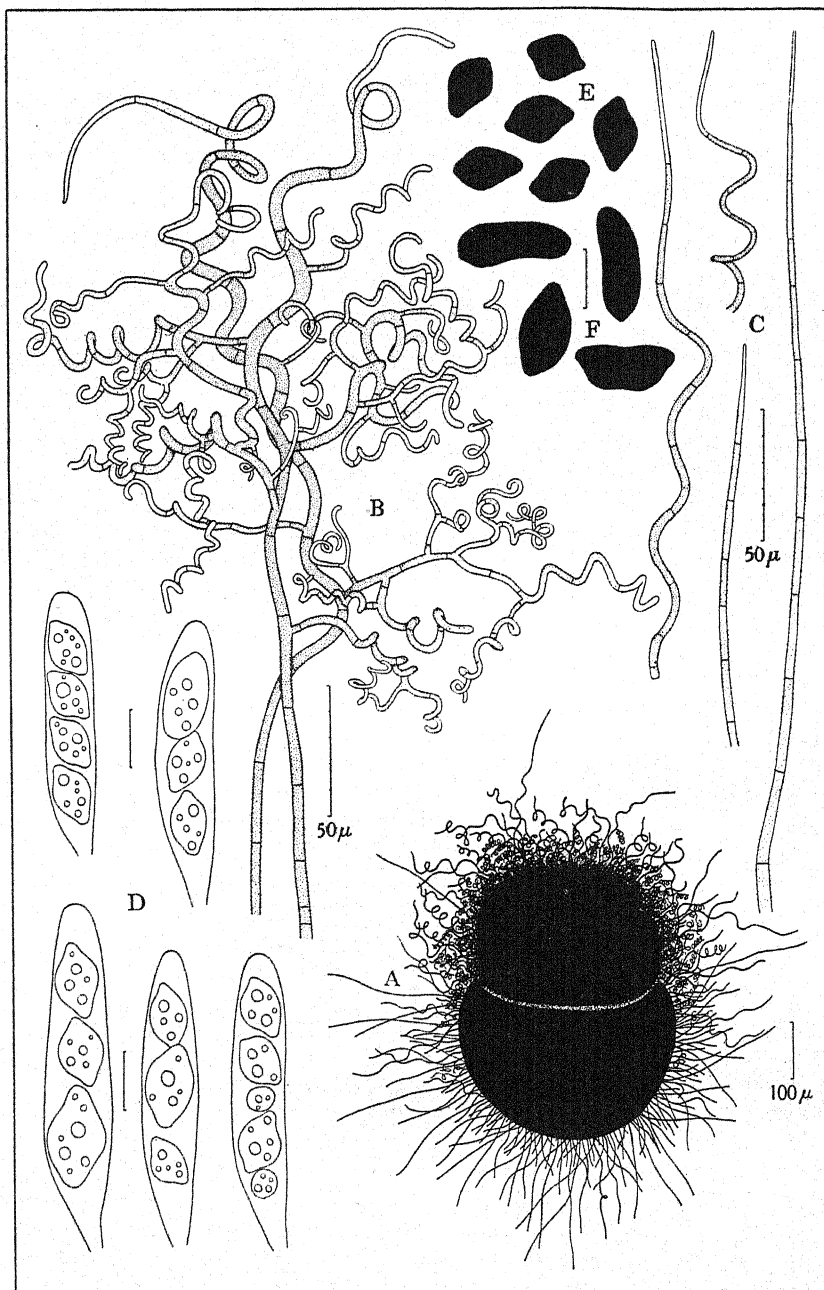


Fig. 1. *Chaetomium tetrasporum*. A, mature perithecium; B, two much branched terminal hairs; C, straight and coiled lateral hairs; D, asci showing young ascospores with oil globules. Asci producing two normal spores with one giant spore, and three normal spores with two dwarf spores are also figured; E, normal ascospores; F, giant ascospores.

Except where otherwise indicated, each scale is 10 μ long.

(a) straight, unbranched, finely roughened, septate, $3.5\ \mu$ wide, pale olive-yellow to brown at base, tapering to hyaline apex; (b) loosely coiled, olive-brown at base, slightly paler at apex, usually unbranched but sometimes with a short, coiled lateral at its base, heavily roughened with minute warts, septate, up to $5\ \mu$ wide; terminal hairs septate, loosely coiled, dark olive-brown and roughened but paler and smoother towards the rounded apex, about $5\ \mu$ wide, bearing conspicuously branched, loosely coiled laterals, whose ultimate branches are narrower, closely and evenly coiled with up to eight convolutions, each coil pale olive-brown or hyaline and almost smooth, with a rounded apex. Asci more or less cylindrical, stalked, $65\text{--}83 \times 7.5\text{--}10.5\ \mu$, pars sp. $45\text{--}52\ \mu$ long, four-spored. Ascospores obliquely uniseriate, at first hyaline, later dark olive, lemon-shaped and strongly apiculate, often angular, elliptical and compressed when viewed edgewise, $10.5\text{--}14 \times 6\text{--}9\ \mu$, mostly $12 \times 7\ \mu$. Paraphyses absent.

Chaetomium tetrasporum Hughes, sp. nov.

Perithecia grisea, subglobosa, ostiolata, $300\text{--}450\ \mu$ diametro, in rhizoidibus male evolutis insidentia. Pili laterales copiosi, nunc recti, haud ramosi, septati, $3.5\ \mu$ diam., sparse asperati, basi ex olivaceo-luteo brunneum sursum hyalini, nunc laxe spiraliter convoluti, basi olivaceo-fusci, $5\ \mu$ diam., sursum pallidiores, vix ramosi, verrucis minutis dense asperulati. Pili terminales septati, ramosi, basi $5\ \mu$ diametro, fortiter asperulati, olivaceo-fusci, sursum pallidiores, ramulis extremis hyalinis; rami majores laxe spiraliter convoluti, ramuli extremi angustati, $3.5\ \mu$ diam., arcte aequaliterque ad 8 spiraliter contorti, dilute olivaceo-brunnei vel hyalini, apicibus fere laevibus. Asci cylindraceo-clavati, basim versus attenuati, $65\text{--}83 \times 7.5\text{--}10.5\ \mu$, pars sporifera $45\text{--}52\ \mu$ tetraspori, aparaphysati. Sporae oblique monostichae, olivaceo-fuscae, limoniformes, utrinque fortiter umbonatae, saepe tetragonae, $10.5\text{--}14 \times 6\text{--}9\ \mu$ (av. $12 \times 7\ \mu$).

Hab. in culturis fimi cuniculorum, et stramenti in laboratorio Cardiff, Cambriae. Nov. 1944.

Chaetomium tetrasporum is a very distinctive species and, apart from its four-spored asci, it can be recognized readily by the great number of small coils comprising the head. The development of the hairs can be observed in young perithecia quite easily as the ascospores take a long time to exude through the ostiole and obscure the view. Indeed, the ascospores are slower developing in this species than in any others kept under observation; this may well be due to the reduced number of spores developing in each ascus. As in many pyrenomycetes with four-spored asci, interesting abnormalities of spore production are seen; giant and dwarf spores are quite common but cultures from single spores have not been made to determine whether ' + ' and ' - ' strains exist.

Terminal hairs from six perithecia have been examined and all show coiling in a counter-clockwise manner (see Davidson & Gregory, 1937).

Dried type isolations have been deposited in the herbaria of the Imperial Mycological Institute, Royal Botanic Gardens at Kew, British Museum

(Nat. Hist.), U.S. Department of Agriculture at Washington, the Central Experimental Farm, Ottawa, and Farlow Herbarium, Harvard University, Cambridge, Mass.

I am indebted to Miss E. M. Wakefield for editing the Latin diagnosis, to Dr S. P. Wiltshire of the Imperial Mycological Institute for providing laboratory facilities and to Dr L. M. Ames for sending the cultures mentioned in the text.

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MYRIANGIUM

By T. PETCH

In 1924 I published an account of the genus *Myriangium*, giving its history and describing the known entomogenous species, after examination of specimens in the herbaria of London and Paris. In 1940 J. H. Miller published a paper, 'The Genus *Myriangium* in North America', which I have only recently (1945) been able to consult.

In his historical review (p. 589), Miller wrote, 'In the United States, Tuckerman in *Genera Lichenum* in 1872 shows that *Myriangium Duriaei* of Europe is equal to *Myriangium Curtisii* of America'. I assume that 'is equal to' means 'is the same as'. But Tuckerman scarcely did that. He certainly suggested that they were 'by no means satisfactorily distinguished' from one another, but he did not treat them as the same. He stated (in error) that both species occurred in Europe, having misinterpreted Nylander and Millardet, but as regards the United States his paragraph on *M. Duriaei* records that 'reaching Cuba, [it] should be likely to appear also within our southern boundaries. And the plant (*M. Curtisii* Mont. & Berk.) which does occur here, and extends northward along the coast (Carolina, Curtis, Ravenel; Alabama, T. M. Peters; Massachusetts, C. F. Sprague, H. Willey),' etc. Thus Tuckerman in 1872 was not aware that *M. Duriaei* occurred in North America; for him all North American specimens of *Myriangium* were then *M. Curtisii*.

After the publication of *M. Curtisii* in 1849, North American specimens of *Myriangium* were assigned indiscriminately to that species, e.g. by Ravenel. Then it was found that some of these specimens were *M. Duriaei*, and hence it was concluded that *M. Curtisii* was merely a large *M. Duriaei*. That idea has been revived by Miller.

In his general account of the genus (p. 590) Miller wrote: 'Petch observed some globose spores in several species of *Myriangium*, but the writer has found only oblong-elliptical ones in parts of the same specimens he examined. The spherical shape appears only in cross sections of spores as shown by the figures of the writer.' I find I gave details of three specimens in which I found globose spores. One of these specimens, Ellis 1279 in Herb. Kew., was not examined by Miller, according to his list, but he examined parts of the same Ellis number in other herbaria, and assigned them to *M. asterinosporum* (Ell. & Everh.) Miller. As, however, he found much larger ascospores than either Ellis and Everhart or myself, and did not find any globose spores, there would appear to be a possibility that Ellis 1279 was a mixture (see p. 76). I did not make use of microtome sections when measuring the asci and ascospores, using them only to determine the arrangement of the asci. To avoid further misconceptions, I may say that the sections shown on Pl. III of my paper were stained.

Figs. 1-4, Pl. II, of my paper are of *M. Duriaei* as it occurs in England. Fig. 6 of the same plate is from a Curtis specimen of *M. Curtisii*, and the

section shown on Pl. III, fig. 4, is from the same specimen. Miller referred the latter to *M. Duriaei*, stating that the section was a typically expanded *M. Duriaei*. I have never seen any herbarium specimen of *M. Duriaei* to match that. It certainly seems strange that although the distribution of *M. Duriaei* extends through temperate and tropical zones, this 'expanded' form occurs only in the United States. Miller recorded that the 'English specimen from Currey on *Fraxinus* is the best developed one of the foreign collections in the New York Botanical Garden herbarium', and elsewhere he listed it as 'Currey, W., *M. Duriaei*, on *Fraxinus excelsior*, April 1876'. It would have been interesting to have had information about the apothecia of that specimen. Should not the name of the donor be W. Curnow?

Under *M. asterinosporum* (Ell. & Everh.) Miller, the latter author wrote: 'This species was designated *M. Curtisii* by the writer (l.c.) in 1938, and also by Petch (l.c.) in part, but recent investigations lead to the conclusion that Montagne had both of the common American species before him and combined the two in his description of *M. Curtisii*.' Miller referred (in part) to my account of the specimens which were under *M. Curtisii* in the Montagne herbarium in 1921, but before proceeding to discuss those again, it may be as well to interpolate a note regarding old-established herbaria which may enable the reader to understand the position.

Specimens acquired by a herbarium are placed in their appropriate place on sheets or in covers (folders) according to some recognized classification. Those which come in unnamed are identified by the staff, but specimens sent in named, or purchased as exsiccata, are usually 'laid in' as named by the seller or sender, as there is rarely time for the staff to verify the identifications and they cannot be specialists in every branch of systematic mycology. Consequently, any given folder may contain more than one species, and the situation is only remedied when some one examines the contents of a folder and sorts out and names the different species.

The type of *M. Curtisii* was collected by Curtis, was sent to Montagne by Berkeley, and was described by Montagne in 1849. The type specimen should be in Montagne's herbarium, and it should be a specimen collected by Curtis during or before 1849. Anything collected after that date or by some other collector cannot be the type.

In the Montagne herbarium in 1921 there were six specimens marked *Myriangium Curtisii*. I number them here for convenience of reference. No. 1 is named '*Myriangium Curtisii* Berk. & Mont.' by Broome. No. 2 appears to be part of the same gathering, and is marked '*Myriangium Curtisii* Berk. & Mont., Car. Inf.' by Berkeley, and 'ex clar. Berkeley, 1853' by Roussel. No. 3 is an American specimen from Sprague. No. 4 is Lindig 2583, Nova Granata. All the foregoing are *M. Duriaei*, and they are all specimens named by other mycologists than Montagne and subsequent to 1849. No. 5 is marked by Montagne '*Myriangium Curtisii* Mont. & Berk., Car. Inf.', and is enclosed in a piece of paper bearing the inscription '*Myriangium*, the best specimen' by Curtis. No. 6, '1442 Car. Inf.' is marked by Montagne '*Myriangium Berkeleyi* Montag., *Myriangium Curtisii* Berk. & Mont. Car. Super. [sic] cl. Berkeley', and matches no. 5.

No. 5 should be taken as the type. It answers to Montagne's description. Note that Curtis was not able to name it except generically, a fact which may indicate that it had not been named when he sent it. Absolute certainty is impossible, as it is not dated, and Montagne did not give any date or collection number in his description.

I see no reason to suppose that Montagne drew up the description of *M. Curtisii* in 1849 from two species. The fact that he cited both Curtis and Ravenel as sources of *M. Curtisii* in *Syll. Plant. Crypt.* (1856) is immaterial. In his original description of 1849 he cited Curtis only.

Berkeley gave a specimen of *M. Curtisii* to Phillips, labelled by Curtis, '*Myriangium Curtisii* B. & M. ad Styracem, fine specimens'. That specimen is now in Herb. Mus. Brit., marked by Phillips, '*ex* Herb. Berkeley'. It matches the Curtis specimen in Herb. Montagne which I take as the type, but it cannot be regarded as a cotype, because Curtis was able to name it, thus indicating that it was sent after Montagne's publication of the name.

Miller stated that in the Farlow Herbarium at Harvard there is a Curtis specimen from Tuckerman's herbarium labelled *M. Curtisii* [? by whom], which should be the cotype. The date and locality of that specimen are not given, but it is not a cotype. It has been pointed out on several occasions that the specimens retained by Curtis are not cotypes. Again, on p. 592, under *M. Duriaei*, Miller listed '*M. Curtisii* Berk. & Mont. E. Tuckerman herb. *ex* Michener, Chester, Pa., 1852: 2 specimens (probably cotype of *M. Curtisii*).' But from the date (1852) those cannot be cotypes of *M. Curtisii* and, as shown above, Tuckerman, until at least 1872, regarded all North American specimens as *M. Curtisii*.

Miller concluded that 'as both descriptions [*M. Duriaei* and *M. Curtisii*] fit *M. Duriaei* [*sec.* Miller], and the Curtis and Ravenel specimens named *M. Curtisii* [in America] are in reality *M. Duriaei*, the Ellis name *asterinosporum* becomes the first to be applied to this species'. That seems somewhat obscure. Presumably 'this species' means *M. Curtisii*; in the section on *M. tuberculans*, Miller wrote '*M. Curtisii* (*M. asterinosporum* of this paper)'. But until Miller has examined the Curtis specimen in Montagne's herbarium he is not entitled to assume that *M. Curtisii* is *M. Duriaei*. That Ravenel's specimens are is true, as far as I have seen them, but that is irrelevant.

Myriangium asterinosporum was described by Ellis and Everhart in 1883 as *Cenangium*. Subsequently they decided that it was *Myriangium Duriaei*. Ellis distributed specimens in North American Fungi no. 1279, of which there are two copies in Herb. Kew. Ellis gave the spores as oblong-elliptic or subpyriform, constricted in the middle, three-septate, submuriiform, $15-20 \times 6-8 \mu$. I examined a Kew specimen and found the spores practically the same as Ellis, $16-19 \times 7 \mu$, and as the stromata were green internally I decided that the fungus was *M. Duriaei*, but with spores smaller than usual. Miller stated that older parts of the Ellis type have spores, $25-34 \times 9-14 \mu$, and suggested that Ellis and myself gave measurements of immature spores. But these were sufficiently mature to show transverse and longitudinal septa. They may have lacked the full number of septa, but one would not have expected them to become almost double in length

and breadth. It is not clear what Miller means by 'older parts of the Ellis type'—presumably older stromata in other copies of no. 1279. He cited as the type of *M. asterinosporum*, Ellis herb. no. 1279. It is possible that the original collection may have been a mixture, but Ellis and Everhart's description agrees with the specimens distributed to Kew.

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(Accepted for publication 28 January 1946)

A DAILY CENSUS OF *ALTERNARIA* SPORES CAUGHT FROM THE ATMOSPHERE AT CARDIFF IN 1942 AND 1943

BY H. A. HYDE, *National Museum of Wales, Cardiff* AND .
D. A. WILLIAMS, *Llandough Hospital, Cardiff*

(With 1 Text-figure)

1. INTRODUCTION

It has for some years been apparent to medical men interested in asthma and allied allergic conditions that a study of air-borne mould spores in Britain was much to be desired.

The earliest suggestion that mould spores might be related to respiratory dysfunction in man appears to be contained in an account by Blackley (1873) of how, following the inhalation of *Penicillium* spores, he himself experienced hoarseness and loss of voice going on to 'bronchial catarrh'. He found the involuntary experiment so unpleasant that he did not care to repeat it of his own accord. In spite of this hint, conveyed in a publication that has become a medical classic, the hypothesis that fungus spores were one of the causes of asthma was not definitely formulated until fifty years later, when it was put forward independently by Van Leeuwen (1924) in Holland and Cadham (1924) in Canada. The genus *Alternaria* was first proved to be implicated by Hopkins, Denham and Kesten (1930), who proved beyond doubt that its spores were responsible for attacks of asthma in a man thirty-seven years of age. They showed by measurements of the spores that the species concerned should be placed in Elliott's *A. tenuis* group (Elliott, 1917). Their report was followed by many others, in which not only individual cases but groups of cases of sensitivity to mould spores were described. It is now accepted in the U.S.A. that mould allergy is of considerable importance there though its exact incidence is still in dispute. Reports suggest that it varies considerably in different localities. According to various authorities quoted by Blumstein (1945) from 3 to 42 % of asthmatics are sensitive to moulds. The genus which most frequently causes symptoms is *Alternaria*.

Mould spores regarded as allergens act in exactly the same way as do pollen grains. They are inhaled and their cell contents are absorbed, giving rise, in persons who are sensitive to them, to a chain of reactions resulting in nasal catarrh and asthma or both. Experience has shown that pollinosis (i.e. hay fever proper) is not caused by any and every kind of pollen: it is most commonly due to certain kinds of pollens which occur widely and abundantly in the air. Following Thommen (1931) therefore, who was dealing with pollen allergy, we may postulate certain requirements in regard to those kinds of fungus spores which are likely to cause asthma: they must contain a specific substance (antigen) capable of

evoking an allergic reaction, they must be buoyant and become easily air-borne, they must be produced in large quantities and they must be of wide occurrence. If these postulates are well founded one would anticipate that, while a certain small number of asthmatics might prove to be sensitive to mould spores occurring occasionally in heavy concentration indoors, a great many more would react to spores occurring regularly in heavy concentrations outside. The physician, then, in his search for fungus spores likely to excite allergic reactions, looks to the mycologist for certain information. He needs to know:

(1) What kinds of mould spore are to be found in the air and in what order of abundance.

(2) Whether the commonest types of spores occur constantly throughout the year or are seasonal in their incidence.

(3) How far the seasonal incidence (if any) of these common types of spore varies from year to year.

(4) Whether, and if so to what extent, spore incidence can be correlated with weather conditions.

The physician requires this knowledge primarily for the district in which he is working; he should also have similar data in regard to other areas, so that he may be able to advise his patients on the desirability or otherwise of a change of residence.

2. STUDY OF ATMOSPHERIC MOULD SPORES IN AMERICA

Extensive studies of mould spores occurring in the atmosphere have been made in the U.S.A. Two methods have been used, sometimes separately, sometimes together, viz. the culture plate method and the gravity slide method.

A. *The culture-plate method*

Petri dishes containing a suitable culture medium are exposed to the air for short periods at intervals throughout the year and the number and types of spores caught are recorded. Usually the time of exposure is so gauged that had it been exceeded more colonies would have developed than could be identified. The intervals between the exposures have varied from daily to bi-monthly.

This method has been used on the most widespread scale by Morrow, Lowe and Prince (1942) on whose behalf plates were exposed bi-monthly for two years at eighteen cities scattered over nine states in the mid-continental region of the U.S.A. They found

(a) that mould spores are widely distributed throughout the central and south-western States;

(b) that total counts of all mould spores tend to be more uniform throughout the year in the south, but are more seasonal in the north, reaching a peak in the autumn; and

(c) that *Alternaria* spores occur more frequently than any other type, *Hormodendrum* coming second in order.

Essentially similar results have been obtained by other workers (Harris, 1942; Blumstein & McReynolds, 1945; Harsh & Allen, 1945). Bernstein and Feinberg (1942), in counts made on plates exposed daily for five years at Chicago, placed *Alternaria* (with 30 % of the total catch) second to *Hormodendrum*; while *Alternaria* counts varied considerably from year to year, they had a marked seasonal tendency, 80 % occurring during the six months June to November inclusive.

B. *The gravity slide method*

This consists in exposing daily for twenty-four hours a glass slide coated with a suitable adhesive; the slide is fixed horizontally in an apparatus by means of which the slide is protected from the weather, but which allows the air to pass freely over it. At the end of the twenty-four hours it is mounted and examined under the microscope, and the spores which have been caught are identified and counted.

This method has been used extensively by Durham (1937, 1938), on whose behalf slides were exposed at sixty-three stations throughout the American Union. The same author (Durham, 1944) has published a tabular summary of the results not only of his own survey but also of other similar observations made throughout North America. He shows that the average seasonal catch on 1.8 sq.cm.* may vary from about 34,000 (in Minnesota) down to fifteen in the Yellowstone National Park and nil in Alaska. High counts (5000 or more) have been obtained at other stations in the Middle West. Counts of between 500 and 1000 were reported from various stations on or near the Atlantic seaboard and relatively low ones (less than 100) in the Pacific States. In general the heaviest incidence was felt during the three months July, August and September, the difference between this period and the rest of the year being more marked in the north than in the south.

It is evident from this brief summary that American studies based on the culture plate method and the gravity slide method both agree that

- (a) *Alternaria* spores occur with great frequency in the air in U.S.A.;
- (b) that they are much more abundant in certain regions than in others; and
- (c) that they exhibit a marked annual periodicity.

3. *ALTERNARIA* SPORES AT CARDIFF

(a) *The daily census of spores, 1942-3*

No studies of air-borne mould spores such as those described above appear hitherto to have been carried out in this country. From November 1941 down to the present time we have exposed gravity slides daily at Llandough

* This is the horizontal area which in theory should receive in 24 hours a number of spores equal to that contained in one cubic yard of air, on the assumption that the atmospheric spore concentration remains constant and that the air itself is non-turbulent. It is now realized that neither of these conditions is ever likely to be observed in nature, but for the sake of convenience of comparison counts in the U.S.A. are still made on, or stated in terms of, 1.8 sq.cm.

Hospital, Cardiff, primarily to catch pollen. In the present paper we describe the results of counting *Alternaria* spores on the slides which were exposed by us during the years 1942 and 1943 only. The site, the vegetational setting and the general procedure have been described in detail elsewhere (Hyde & Williams, 1944).

Llandough Hospital is situated in the country, at an altitude of 200 ft. $3\frac{1}{2}$ miles from the centre of the city of Cardiff and 2 miles from the Bristol Channel. It is estimated that in 1942 the land within a radius of $1\frac{1}{2}$ miles was utilized as follows:*

	%	
Grassland	45.5	
Straw crops	20.0	
Other arable land	4.5	%
Total cultivable land		70
Woodland		10
Urban and estuarine land		20
		<hr/> 100

In 1943 the acreage under straw crops (then at its war-time peak) probably rose to 22 %. Wheat made up 55 % of the cereal acreage, oats 35 % and barley 10 %.

The procedure may be briefly stated thus: a 3×1 in. microscope slide which had been previously coated with a thin covering of melted glycerin jelly was put out every morning at 10 a.m. in an apparatus (situated on the Hospital roof, 22 ft. above ground) which allowed a free flow of air to pass over it but which protected it from rain. After a twenty-four hour exposure the slide was removed and mounted with a $\frac{7}{8}$ in. cover-slip. The whole area (5 sq.cm.) covered by the cover-slip was later examined microscopically.

All the slides exposed during 1942 and 1943 have been examined and the number of *Alternaria* spores seen has been recorded. Those on the 1942 slides have also been measured and co-ordinated.

The identification of our spores as those of *Alternaria* spp. has been based mainly on the descriptions and figures published by Wiltshire (1933). We have submitted some of our gravity slides to Dr Wiltshire. He agrees that the spores which we have entered as *Alternaria* do belong to that genus, though many of them, unlike those of typical *A. tenuis*, do not possess an apical scar. We have also submitted to Dr Wiltshire a preparation made from a culture plate exposed at Llandough on 20 July 1943: he reports that the species concerned is one of the *A. tenuis* group. We have disregarded under-developed and otherwise doubtful spores and our figures are probably conservative.

The results of our daily counts for the two years are summarized, together with data on the average length of the spores caught in 1942, in Table 1, and illustrated graphically in Figs. 1 and 2, in which maximum and minimum temperatures, sunshine, wind and rainfall recorded daily at

* Information on cultivable land from Mr Brynmor Rees, Crops Officer, Glamorgan War Agricultural Executive Committee.

Table 1. *Alternaria* spores caught on 5 sq.cm. at Llandough Hospital near Cardiff

Month	1942			1943	
	No. of spores caught	Maximum no. on one day	Average length of spores (μ)	No. of spores caught	Maximum no. on one day
Jan.	0	—	—	5	3
Feb.	2	1	—	1	1
Mar.	4	2	33.6	1	—
Apr.	17	3	43.3	0	1
May	6	2	47.5	12	—
June	45	5	46.1	24	—
July	148	24	49.0	209	—
Aug.	330	60	45.5 (of 300)	377	—
Sept.	123	31	48.5	116	—
Oct.	34	11	50.5	4	—
Nov.	10	3	38.9	0	—
Dec.	6	2	46.0	0	—
Total	725	—	—	749	—

Penylan, Cardiff have also been entered.* The two curves are essentially similar and in fact the broad agreement between them is remarkably close. They appear to show:

(1) that *Alternaria* spores form a normal and regular part of the annual spore rain at Cardiff;

(2) that while they are virtually absent during the late autumn and winter months, they are present in the spring and summer and in greatest numbers during the months June–September inclusive: the catch during these months totalled 646 spores (89 % of the total) in 1942 and 726 (96.5 %) in 1943;

(3) that the period of highest incidence (June–August) coincides with that of the highest mean temperature; and

(4) that the catch during June–August varies very greatly from day to day. Probably these oscillations are connected in some way with the weather though the exact relationships are not obvious.

(a) There is some correlation, though an incomplete one, between daily catch and maximum temperature.

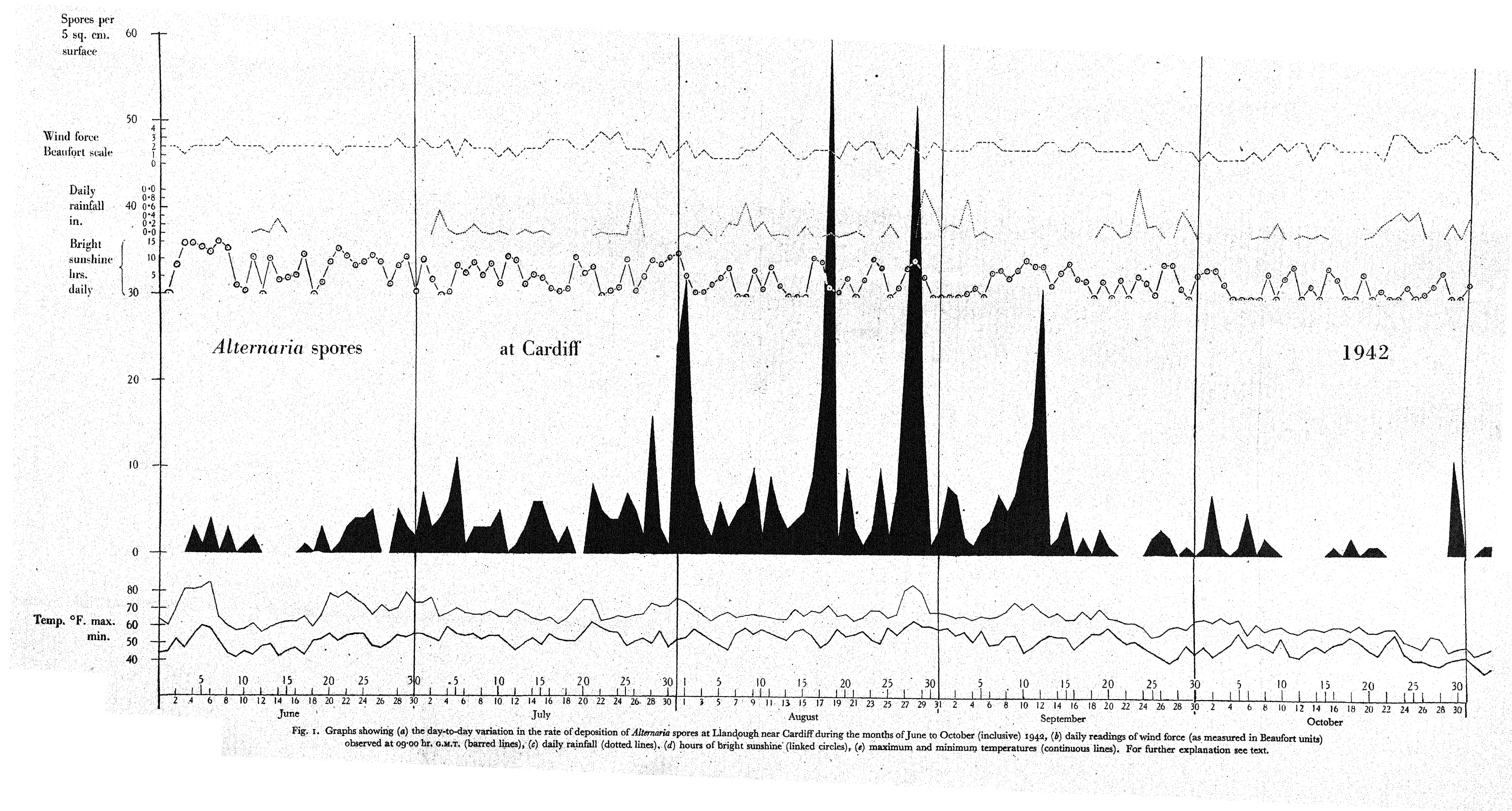
(b) There is no apparent correlation between wind velocity and *Alternaria* catch.

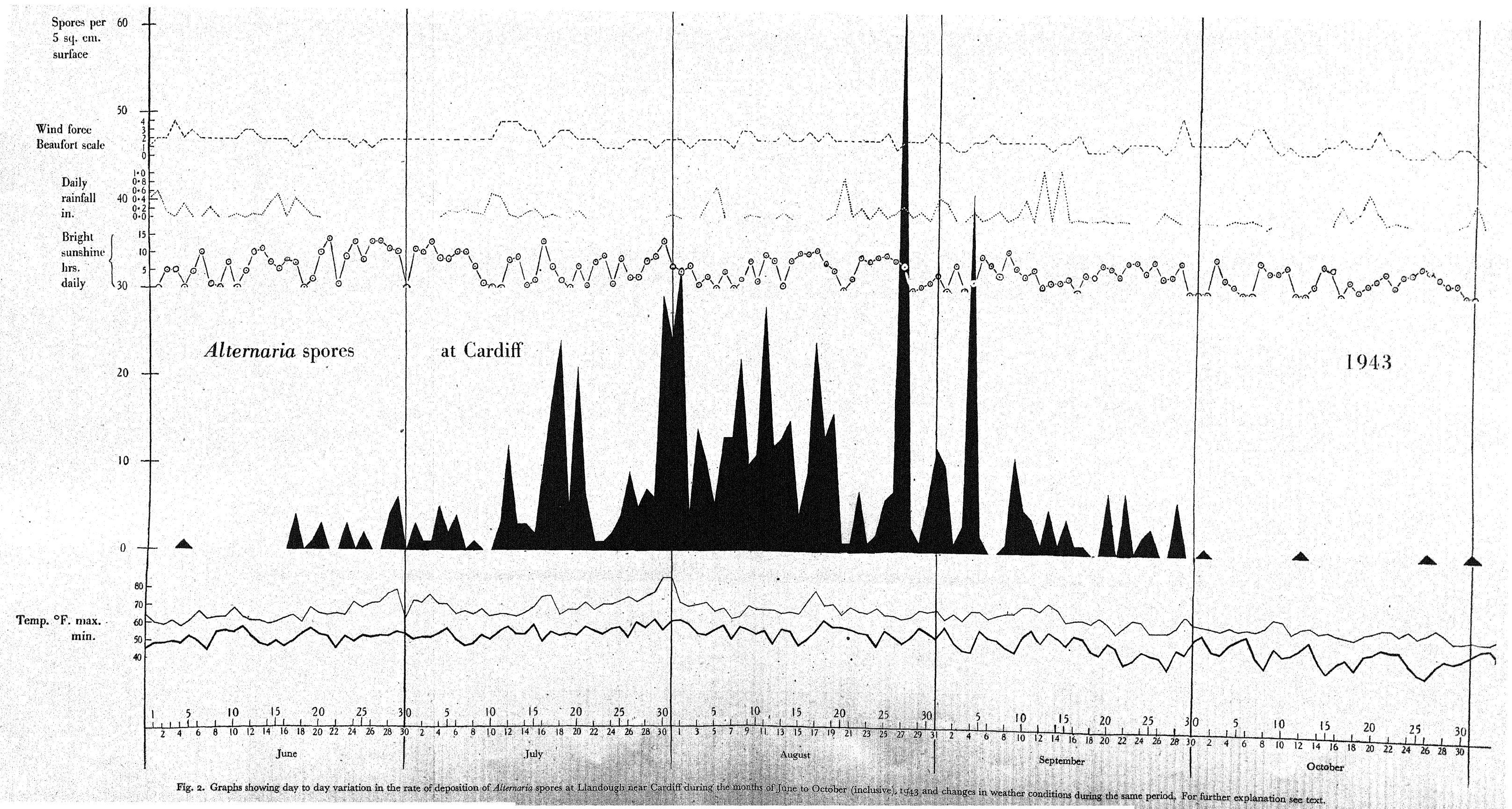
(c) Rainfall on certain occasions seems to have resulted in a marked though temporary diminution in the catch, e.g. on 21–22 August 1943.

It should be added that the rapid falling off in the catch during September may be due (as is suggested below) less to the effect of falling temperature on rate of spore production than to removal of the substrate on which the fungus mainly flourishes.

A comparison of our figures with those in Durham's table (Durham, 1944) shows that the rate of deposition of *Alternaria* spores at Llandough is

* The weather records, which have been supplied by the courtesy of the City of Cardiff Public Health Department, were made at the Meteorological Station, Penylan, which is situated at 202 ft. about $4\frac{1}{4}$ miles north-north-east of Llandough Hospital.





comparable with that at certain stations in the eastern U.S.A. Thus the maximum daily catch and average seasonal total on 1.8 sq.cm.* respectively at the centres named were as follows: Greenville (Maine), 25 and 278; McKeever (N.Y.), 21 and 350; Yorke (Maine), 16 and 202; as compared with Llandough (Wales), 22 and 255.

(b) *The probable source of supply of the spores*

Mortensen many years ago (1910) noted *Alternaria* as being common in Denmark on moist straw of *Avena*, *Triticum* and *Hordeum* (Lind, 1913, p. 536).† Durham (1938) remarked that the straw of wheat and other cereals seems to form an ideal culture medium for *Alternaria*, and thought that the varying incidence of its spores in the U.S.A. both in time and space could thus be accounted for. Ogilvie (1943) reported that the blackening of wheat ears, 'a common and well-known trouble in the western province' (i.e. in the west of England) was due *inter alia* to *Alternaria* spp., which, he informed us, occur very commonly on wheat so affected. As already stated, about 20 % of the land within 1½ miles of Llandough Hospital was under straw crops, largely wheat, in 1942-3. Blackening of wheat ears was very prevalent in the area around Llandough in both the seasons concerned‡ and, furthermore, the date of completion of the wheat harvest (early October in both years) coincided closely with the almost complete disappearance of *Alternaria* from our slides in each of the two years.

We have no means of confirming directly the prevalence of *Alternaria* on wheat at Llandough during 1942 and 1943, but that it does so occur locally is proved by observations made by us in 1945. We exposed prepared slides during threshing operations at Llandough Farm (Mr Thomas, ½ mile from the Hospital) on 2-3 November 1945 and at Ynyston Farm, Leckwith (Mr Coles, one mile from the Hospital) on 31 December 1945. The slides were so placed as to catch the greatest possible amount of dust from the machinery; they were exposed for periods varying from four to twenty minutes. The catch per minute on 5 sq.cm. on 2-3 November, from a stack of Wilhelmina wheat, varied from twelve to eighty-seven *Alternaria* spores (nine slides in all, average thirty-two per minute), that on 31 December, from a stack of Bersée wheat, amounted to seven and nine per minute respectively on the two slides exposed.

The occurrence of *Alternaria* spores on the slides we exposed in 1942 and 1943 might therefore be explained on the hypothesis that they were formed in and liberated from wheat and other cereal fields around the Hospital. That they should have been derived from local sources is what in general we should expect from our work on atmospheric pollen: a daily catch of fifty grains or more of a particular type on 5 sq.cm. can usually though not always be related to a probable source within a mile of the observation station.

* See footnote, p. 80.

† We are indebted for this reference to Mr John Rees.

‡ Information from Mr Brynmor Rees, Crops Officer.

4. *ALTERNARIA* SPORES IN RELATION TO ALLERGY

We do not at present know to what extent our findings apply outside the Cardiff area. We hope later, by the help of slides which during the past few years have been exposed for us in various parts of Great Britain, to extend our observations. In the meantime it is clear that around Cardiff at certain times the atmospheric concentration of *Alternaria* spores is sufficiently high to create a presumption that *Alternaria*-spore allergy exists among the human population, and that catarrhal or asthmatic attacks occurring either during the late summer, especially near wheat fields, or at any other time near the scene of wheat threshing, may fairly be suspected of having some such origin.

5. SUMMARY

1. The daily incidence of *Alternaria* spores at Cardiff in 1942 and 1943, as determined by counts made on gravity slides, is described.
2. It is shown that, during the two years concerned, the deposition of *Alternaria* spores was virtually confined to the period June to September inclusive.
3. The relation of *Alternaria*-spore incidence to various atmospheric factors is illustrated diagrammatically and briefly discussed.
4. The provisional hypothesis is advanced that the *Alternaria* spores caught at Llandough originated mainly on cereals growing in the vicinity.

We desire to thank Dr D. G. Morgan (Medical Superintendent, Llandough Hospital) for his personal encouragement of this investigation.

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NATURAL OCCURRENCE OF SPROUTING OF THE ASCOSPORES OF *SPHAERULINA MAPPIAE* (PETCH) N.COMB., IN CEYLON

By T. E. T. BOND, *Tea Research Institute of Ceylon*

(With Plate IV)

DESCRIPTION

The fungus under consideration, originally described as *Sphaerella mappiae* Petch (1922), is a little-known species causing a leaf-spot of the shrub *Mappia ovata* Miers in Ceylon. This host occurs sparingly on the Tea Research Institute's estate,* and two or three plants, kept under observation for more than a year, have remained continuously and very heavily infected over the whole period.

The symptoms (Pl. IV, fig. 1) are striking. Petch (1922) briefly describes the spots as 'pale brown, circular, up to 2 cm. diameter, often irregularly zoned'; in the fresh condition, however, a bizarre effect is produced by the differences in colour between successive zones and their irregular juxtaposition, many of the zones being incomplete. The centre of the spot is almost white, with the surrounding zones of successively darker shades of brown. The zones are separated from one another by slightly raised, wavy margins; the outermost impinges directly on the unaltered green tissues, or there may be a narrow, purplish border. The differences in colour are most pronounced on the upper surface.

The perithecia are entirely hypophyllous, crowded but quite separate, immersed, erumpent, averaging about 125μ in diameter. The asci (Pl. IV, fig. 2) are clavate, 50μ or more in length, and contain eight biserial spores. The latter, while still within the ascus, though ripe in appearance and readily extruded in water, are entirely uniseptate. A sample of twenty-five ascospores, in this condition, measured $15.5-21.0$ by $2.5-4.0\mu$, with a mean of 17.9 ± 0.3 by $3.7 \pm 0.1\mu$. The spores are straight or slightly curved, usually somewhat clavate with the lower cell narrower and more pointed than the upper. The septum, which is marked by a slight constriction, is almost invariably median.

The characters described so far agree sufficiently well with Petch's (1922) diagnosis of *Sphaerella mappiae*, while examination of the type material, Herb. Perad. 5236, although unfortunately revealing immature asci only, with no spores, confirmed the specific identity of the two collections. The chief interest, in the present instance, lies in the further development of the ascospores. In the field, in the early part of 1944 and again in 1945, in both cases during a period of dry weather, most infected leaves bore extruded spore masses adhering *in situ* around the emergent ostioles. The masses

* Near Talawakelle, C.P., elevation c. 4500 ft.

were yellowish, firm and coherent in texture, not easily manipulated without crushing. They were found (Pl. IV, fig. 3) to consist of enlarged, two-, three-, and four-celled ascospores with very numerous sprout cells produced terminally and laterally by the constriction of a narrow sterigma or neck. The sprout cells are bacillar, oblong or somewhat narrower below, straight or very slightly curved, apparently biguttulate later, in size $4.5-7.0$ by $1.5-2.5\mu$ with a mean (25) of 5.4 ± 0.14 by $2.0 \pm 0.05\mu$. Their adhesion in a compact mass with the original ascospores indicates that they are classifiable (Mason, 1937) as 'slimy radula spores'. They were not observed to germinate. The associated ascospores varied in size from $14.5-30.5$ by $3.5-6.5\mu$ with a mean (50) of 22.3 ± 0.5 by $5.2 \pm 0.03\mu$. One or both of the original cells may be enlarged, and septate, the additional cross walls not being associated with any constriction. The production of sprout cells is not confined to the spores which have become multiseptate nor, apparently, is it necessarily an immediate consequence of this condition. In water, the ascospores in all conditions have been observed to germinate only by the production of apical germ tubes; presumably, the budding process takes place in moist air, as is suggested also by the increase in size and abundance of the adherent spore masses on leaves placed in a moist chamber.

DISCUSSION

The production of sprout cells and other instances of 'fructificative germination' (Brefeld, 1891) in ascospores was a favourite subject of investigation among the earlier authors. It is well known to occur in the yeasts, in the Taphrinales and certain Hypocreales (de Bary, 1887), and in such forms as *Plowrightia* (*Dothidella*) *ribesia* for which Hoggan (1924) investigated the possibility of a connexion with *Dematium pullulans*. It was reported in the Erysiphales by Paunero Ruiz (1927). Generally, however, it has received scant attention from later writers, particularly in the group to which the present species belongs, namely, the Sphaeriales, and 'Mycosphaerellaceae' in particular. Bertus (1927) described a rather unusual instance of the abscission of conidia from the ascospores of *Glomerella piperata* in Ceylon, but the best-known example is that of *Sphaerulina intermixta* described and illustrated by Brefeld (1891). (For modern views on the nomenclature of the fungus, see Bisby and Mason, 1940.) Brefeld's account, with his figure, is reproduced by Gäumann (1926) and, with some reserve, by Gäumann and Dodge (1928). The condition is identical with that now described, from nature, for the fungus on *Mappia ovata* and it is interesting to find the original observation vindicated.

The biological significance of the phenomenon cannot profitably be discussed in the absence of experimental data. But the very heavy degree of infection invariably encountered with the *Mappia* leaf-spot suggests that the sprout cells are an important and effective means of dispersal.

There remains the question of the generic position of the present species, namely, whether the eventually multiseptate condition of the ascospores warrants a transfer from *Sphaerella* Ces. & deNot. to *Sphaerulina* Sacc. That it does so would seem to be in accordance with accepted tradition. More-

over, while I have found no evidence in the literature for the development of additional septa in the ascospores of *Sphaerella* (incl. *Mycosphaerella*) spp., there has been no lack of evidence for the long persistence of the uniseptate condition in *Sphaerulina*. *S. taxi* Massee (1915) will probably be the most familiar example (Callen, 1938); at least seven other species can be included on the basis of their diagnoses in Saccardo. Finally, the budding of the ascospores can itself corroborate the proposed transfer in that the process as here described appears to be unknown in other species of *Sphaerella*, for which genus Brefeld (1891) instances a quite distinct type of 'fructificative germination' leading to the formation of *Hormodendron*-like chains of relatively large, acropetal spores (Pl. IV, fig. 4) which are evidently thallose in the sense of Mason (1937). As already indicated, I am satisfied as to the specific identity of the present fungus with the type of *Sphaerella mappiae* Petch. Accordingly, the transfer to *Sphaerulina* necessitates a new combination, which is now formally proposed. On the basis of the additional information now available, the diagnosis of the species (in English originally) is amended and a new collection, showing the *Sphaerulina* condition, is specified as a homoeotype. In the amended diagnosis, the writer's additions are printed in italics:

Sphaerulina mappiae* (Petch) Bond, *n.comb.

Sphaerella mappiae Petch, *Ann. R. bot. Gard., Peradeniya*, vii, 303 (1922)

'Spots pale brown, circular, up to 2 cm. diameter, often irregularly and prominently zoned. Perithecia hypophyllous, crowded, immersed, about 0.1 mm. diameter. Asci clavate, narrowed above, thick-walled, almost sessile, spores biserial, $36-40 \times 12-14 \mu$, becoming larger later; no paraphyses. Spores narrow-oval or fusoid, becoming somewhat clavate, hyaline, at first one-septate, later mostly two- to three-septate, $12-30 \times 2.5-6.5 \mu$, capable of producing abundant sprout cells.'

On leaves of *Mappia ovata* Miers, in Ceylon.

Type: Herb. Perad. 5236, Hakgala, April 1917.

Homoeotype: Herb. T.R.I. 350, St Coombs, Talawakelle, Feb. 1944.

Duplicates of the homoeotype have been sent to Peradeniya, New Delhi, and the Imperial Mycological Institute, Kew.

In conclusion, it is worth noting that no conidial stage of this fungus has as yet been detected. *Septogloeum mappiae* Petch (1917), collected originally from the same locality as '*Sphaerella mappiae*', is suggestive in this connexion, but does not appear to occur at St Coombs. Examination of the type of the former species, Herb. Perad. 4730, has revealed no trace of any associated perithecial stage.

SUMMARY

A leaf-spotting fungus identifiable with *Sphaerella mappiae* Petch is described on *Mappia ovata* Miers in Ceylon. In two successive years the perithecia were observed with adherent, extruded spore masses in which the majority of the ascospores had become two- to three-septate and had budded off large numbers of 'sprout cells'. The significance of this be-



Fig. 1.

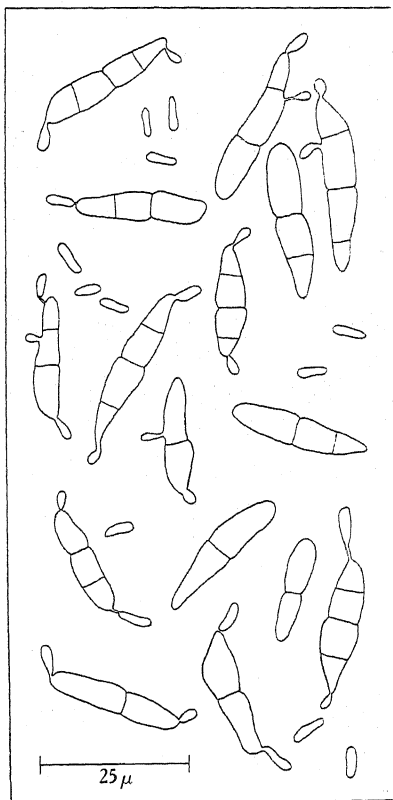


Fig. 3.

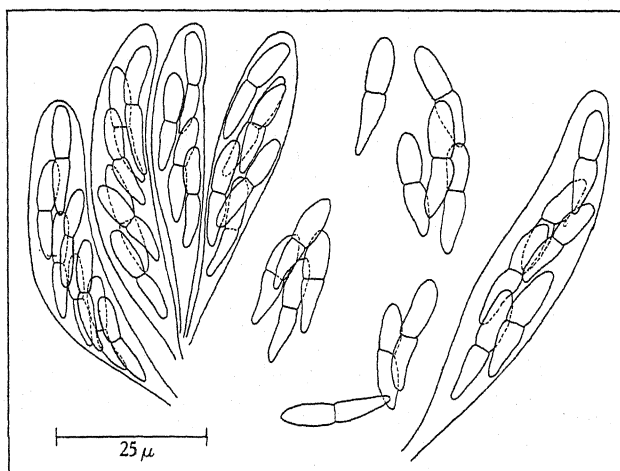


Fig. 2.

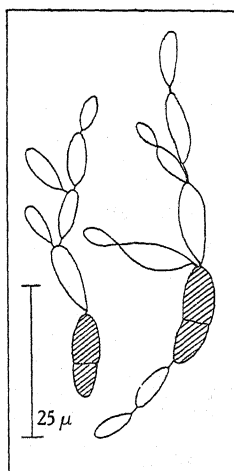


Fig. 4.



haviour is discussed, with the conclusion that the species should be transferred to the genus *Sphaerulina*. The new combination *Sphaerulina mappiae* (Petch) Bond is formally proposed, the diagnosis being slightly amended and a new homoeotype specified. No connexion has been found with the conidial fungus *Septogloeum mappiae* Petch, on the same host.

Grateful acknowledgment is due to Dr S. P. Wiltshire for advice on taxonomy.

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EXPLANATION OF PLATE IV

- Fig. 1. Leaf of *Mappia ovata* Miers, showing natural infection by *Sphaerulina mappiae*, $\frac{2}{3}$ nat. size. (Photo by C. A. Loos).
 Fig. 2. Group of asci and ascospores of *Sphaerulina mappiae* obtained from crushed perithecium, in water. Camera lucida drawing, $\times 800$.
 Fig. 3. Mature ascospores and 'sprout cells' of *Sphaerulina mappiae* from extruded spore mass, in water. Camera lucida drawing, $\times 800$.
 Fig. 4. Part of Brefeld's (1891) Taf. vi, fig. 38, showing 'fructificative germination' of *Sphaerella punctiformis* ascospores, in moist air. The original spores are hatched. The figure is reproduced at $\times 800$.

(Accepted for publication 8 February 1946)

NEW AND INTERESTING PLANT DISEASES

By W. C. MOORE, *Plant Pathology Laboratory, Harpenden*23. LEAF BLOTCH OF ALLIUM (*HETEROSPORIUM ALLII* ELLIS & MARTIN AND *H. ALLII* VAR. *CEPIVORUM* NICHOLAS & AGGÉRY)

Four species or varieties of *Heterosporium* have been described on *Allium*, and two of them have been reported occasionally in England.

Ellis and Martin (1885) erected *H. Allii* for a fungus occurring on *Allium vineale* in North America and gave it the following diagnosis: '*Heterosporium Allii* E. & M.—on withered leaves of *Allium vineale*, Newfield, N.J. Aug. 1883. Hyphae erect, subcontinuous, nodulose, olive-brown, about $50 \times 9 \mu$. Conidia oblong, fuscous, minutely echinulate, 1–3-septate, $20\text{--}33 \times 9 \mu$. Differs from *H. Ornithogali* in its olivaceous colour and smaller conidia.'

This species has since been reported (Rostrup, 1902 *a*) on shallot (*A. ascalonicum* L.), on garlic (*A. sativum* L.) and (Rostrup, 1902 *b*) on chives (*A. schoenoprasum* L.) in Denmark; on garlic in Moravia (Baudyš, 1930); on leek (*A. Porrum* L.) in Belgium (Marchal, 1938) and Canada (Connors, 1944); on chives in England; on various wild species of *Allium* in the United States (Overholts, 1934; Jacques, 1941); and in soil in India (Chand, 1937). In Rab. *Krypt. Fl.* 1, ix, 78, it is recorded also on *A. fistulosum* as well as on *Lilium lanceolatum* and *Tritona uvaria*. There appears to be no record of it on onion (*Allium Cepa* L.). Jørstad (1945) has recently listed *H. Allii* in Norway on the leaves of onion, shallot, leek, chives and *Allium fistulosum*, and *H. Allii-cepae* (syn. *H. Allii* var. *cepivorum*) on the leaves and stems of *Allium fistulosum*.

Briard (1886) found a fungus in France on dead or dying leek leaves, and named it *Heterosporium Ornithogali* Klotsch. var. *Allii Porri* Sacc. & Br., but later the same year Saccardo (*Syll. Fung.* iv, 480) listed it as *H. Allii* var. *Allii-Porri* Sacc. & Br. The conidiophores were torulose, septate, $80\text{--}120 \times 8\text{--}10 \mu$, with greyish brown, 1–3 septate conidia $28\text{--}44 \mu$ long and $12\text{--}16 \mu$ broad. The only other record of this variety I have found is one on *Allium* in Pennsylvania in 1929 by Overholts (1934), who gave the spore measurements as $42\text{--}66 \times 12\text{--}15 \mu$.

Ranojević (1910) was the first to report a *Heterosporium* on onion. He found it present in grey spots on wilting onion plants in Serbia in June 1905, and named it *H. Allii-Cepae* Ranojević. The conidiophores were up to 198μ long and $7.5\text{--}20 \mu$ broad. The conidia were either 1-celled, pear-shaped, club-shaped or long elliptical, $32\text{--}48 \times 9.5\text{--}19 \mu$, or 1–2, occasionally 3-septate and then elongated, straight, often narrower in the middle and pupa-like, $44\text{--}76 \times 9.5\text{--}20 \mu$ (or even up to $101 \times 25 \mu$). I am not aware that this species has been reported elsewhere.

Nicholas and Aggéry (1927) also found a *Heterosporium* associated with a leaf disease of onions in France, and called it *H. Allii* Ell. & Mart. var.

cepivorum n.var. They regarded it as distinct from all the known species of *Heterosporium*, including *H. Allii-Cepae*, though I see no good reason for distinguishing it from Ranojević's fungus. *H. Allii* var. *cepivorum* has brown, nodular conidiophores, very occasionally septate, $40 \times 7.5-10 \mu$ when young, $90-115 \times 7.5-10 \mu$ when mature, bearing 1-3 conidia. The conidia are cylindrical, unicellular or 1-3-septate, mostly 2-celled, and measure $55-100 (-120) \times 10-12.5 \mu$.

Occurrence in England. *Heterosporium* was first noted on *Allium* in Britain in July 1931, when Salmon and Ware (1932) observed a fungus closely agreeing with *H. Allii* var. *cepivorum* on the leaves of five different varieties of onion grown at Whiteparish, near Salisbury. I had an opportunity of examining part of this material. The conidiophores were mostly well within the range given by Nicholas and Aggéry (1927) for *H. Allii* var. *cepivorum*, but a few were over 200μ long and distinctly septate. The spore dimensions were $39-110 \times 9-13 \mu$, with an average of $81 \times 11 \mu$ for thirty conidia, and the fungus might therefore equally well have been identified as *H. Allii-Cepae*. The same variety was observed on onion in Devon in 1939 (Moore, 1943), 1943 and 1945, and is evidently not uncommon in West Cornwall and certain parts of Devon, both on autumn- and spring-sown onions.

In September 1934 Dr W. M. Ware sent me some chives from Crowborough, Sussex, the leaves of which were attacked by Rust (*Puccinia Porri* (Sow.) Wint.) and, in addition, showed a species of *Heterosporium*. When examined after keeping the leaves for a day or two in a moist dish, some of the conidiophores of the *Heterosporium* were over 100μ long, but though many conidia were measured, none was longer than 38μ . They were 1-3-septate, with a range of $25-38 \times 8-12 \mu$ (Salmon & Ware, 1935). It was therefore concluded that the fungus was distinct from the one observed on onion three years before and agreed more closely with *H. Allii* Ell. & Mart.

The only other known locality for *Heterosporium* on *Allium* in Britain is at Tilgate, Sussex, where Dr J. R. Booer and I found it in June 1945 associated with extensive leaf blotching among some autumn-sown White Lisbon onions growing in an old walled-in garden. One or more elliptical, sunken, pale-brown blotches, up to $1\frac{1}{2}$ in. long and about $\frac{1}{4}$ in. wide, were present on many of the leaves. When young they were clearly defined, but as they became larger, and especially where there were several on the same leaf, all the distal portions of the leaves and the tissues surrounding the blotches became pale, or turned yellow and withered. Conidiophores and conidia of *Heterosporium* developed first as a pale, powdery mass in the centre of the blotch and later as a more extensive brown or deep brown mat. The conidiophores were olive-brown, more or less rigid, emerging from the stomata singly or in fascicles of two or three, mostly septate, nodular, slightly swollen at the tip, and measured $90-120 \times 5-6 \mu$. The conidia were pale olive-brown, verrucose, and very irregular in size and shape, frequently 1-celled and then usually pear shaped, but mostly 1-septate and straight-cylindrical or with one cell broader than the other, and occasionally 2- or even 3-septate. The measurements of the conidia varied greatly. Thirty-two conidia taken from young blotches showing the pale, powdery mass

averaged $58-16\mu$: of these seven were 1-celled with a range of $23-60 \times 12-21\mu$ (average $42 \times 16\mu$), while the other twenty-five were all 1-septate conidia and measured $45-75 \times 11-20\mu$ (average $62 \times 15.5\mu$).* The first twenty conidia measured from blotches showing the older brown mat were $37-106\mu$ long (mostly over 70μ), with an average length of 74μ .

Discussion. Jacques (1941), in reporting on his morphological and cultural studies with seven species of *Heterosporium*, said of *H. Allii* Ell. & Mart. that 'the dimensions given in the original diagnosis should not be considered as exact, for it was found in examining the New Jersey material that the length of the conidia exceeds considerably that given by Ellis and Martin'. Jacques gave the dimensions of the conidiophores of this fungus as $19-200 \times 5-12\mu$, and of the conidia $16-70 \times 7-18\mu$ (averaging $37.3 \times 11.6\mu$). A dried Petri dish culture of *H. Allii*, collected by Jacques on *Allium vineale* in Illinois in 1939 and preserved in Herb. Kew., shows conidiophores over 100μ long and conidia up to $60 \times 12\mu$. There are also in Herb. Kew. two specimens of *H. Allii*, both collected on *Allium oleraceum* on 5 June 1931 from the same locality in Latvia. In one of them, from Herb. K. Starcs, I found no conidia longer than 33μ ; in the other, from Herb. J. Smarods, they were up to $40 \times 9\mu$ and the conidiophores up to 100μ long.

As pointed out by Jacques (1941), a number of varieties of *H. Allii* have been recorded on a few genera of the Liliaceae and Amaryllidaceae other than *Allium*. Jacques cited var. *Bomareae* Pat. on *Bomarea*, var. *Polygonati* Oud. on *Polygonatum* and var. *Funkiae* on *Funkia*, and regarded retention of these European varieties as unnecessary, on the ground that they differ from the type and from one another 'only in unimportant variations in degree of development due to the environment or to their occurrence on different susceptibles'. This view may be justified, for none of the varieties named is described as having conidia more than 50μ long, but the same is not true of the fungus on onion. Although Jacques found conidia of *H. Allii* up to 70μ long, the average length was only 37.3μ , which is less than the average length of even the 1-celled conidia, and considerably less than the average length of the septate and more mature conidia in the material from Tilgate. Moreover, the conidia from chives in this country in 1934 were consistently short. It is true that the spore dimensions of many fungi vary considerably with age and with the atmospheric conditions under which the material is collected, and that spore measurements alone do not always provide a reliable basis for distinguishing between species or even varieties, yet the differences shown in this respect by *Heterosporium* on onion and on other species of *Allium* are so marked as to justify, for the time being, a distinction of varietal rank between *H. Allii* Ell. & Mart. on leek, shallot, chives and garlic, and the form on onion, for which the name *H. Allii* Ell. & Mart. var. *cepivorum* Nicholas & Agg  ry is the valid one. *H. Allii-Cepae* Ranojevi   is considered to be synonymous with *H. Allii* var. *cepivorum*, and *H. Allii* var. *Allii-Porri* with *H. Allii*.

* As most of the conidia were distinctly 'waisted' it should perhaps be made clear that the width measured was invariably the greatest width.

24. LEAF SPOT OF *HELIANTHEMUM VULGARE* GAERTN. (*SEPTORIA CHAMAECISTI* VESTERGR.)

In July 1945 Miss J. M. Gooby drew my attention to spotting on the living leaves of certain hybrids of *Helianthemum vulgare* (rock rose) growing in a private garden in Harpenden. The spots were most evident on all plants of a pink variety and were seen also on a narrow-leaved variety with double red flowers, but an adjacent yellow variety was free from them. Most of the spots were not more than 2 mm. in diameter. They were epiphyllous though faintly visible from below, one to many on individual leaves, rounded, reddish-purple or with a pale brown or buff centre, and at times coalescent. Occasionally the spots were as much as 5 mm. in diameter, and then straw-coloured with a narrow purple margin. Similar spots were seen on a few of the sepals.

Pycnidia of a species of *Septoria* were present on a number of the leaf spots: they were very few in number, epiphyllous, sunken and scarcely visible under a lens, 60–90 μ in diameter, with a thin dark brown or blackish wall and no obvious ostiole. The pycnosporos were straight or curved, hyaline, rounded at the ends, guttulate, $15\text{--}39 \times 1.5\text{--}3 \mu$, and doubtfully septate or perhaps with 1–3 septa. The average length of fifty spores was 25 μ . The fungus agreed sufficiently well with the description of *Septoria Chamaecisti* Vestergr. to be identified with that species, which was described on living leaves of *Helianthemum Chamaecistus* Mill. (*H. vulgare*) in Sweden (Vestergren, 1896) and has apparently not been observed elsewhere. *Septoria Helianthemis* Gz. Frag. (*Deuter. Esp.* 1917, 24, Sacc. *Syll. Fung.* xxv (1931), 41) recorded on *H. montanum* in Spain, forms pycnidia with long papillate ostioles on the undersides of the leaves and the spores are much shorter (14 μ), while in *S. Helianthemis* (Vestergr.) Allesch. (*Rab. Krypt. Fl.* 1, 6 (1900), 791), found on rotten, fallen leaves of *Helianthemum* in Sweden, the pycnidia are not produced in spots and the spores are longer (40–60 μ). It is of interest that Grove (*Coelomycetes*, 1, 21) reported finding many *Septoria* spores which 'could hardly be anything else than *Septoria Helianthemis* (Vest.) Allesch.' in pycnidia of *Phyllosticta helianthemicola* Allesch. found on dead leaves, sepals and petals of *Helianthemum* in Ayrshire.

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(Accepted for publication 15 November 1945)

PROCEEDINGS

Meeting held in the Department of Botany, University College, Cardiff, at 10.30 a.m., 20 October 1945. The President, Mr G. Smith, in the Chair.

The Phycomycete Flora of South Wales

W. R. IVIMEY-COOK. Introduction.
Miss E. MORGAN. The Saprolegniales.
Mrs E. A. V. DAVIES. The Pythiaceae.
Miss E. S. L. JONES. The Chytridiales.
Mrs P. E. PERROTT. The Monoblepharidales.
Miss W. DAVIES. The Mucorales.
Discussion.

The Fungus Flora of the Air

R. C. McLEAN. The aerial Micro-organisms.
D. A. WILLIAMS and H. A. HYDE. A daily census of atmospheric *Alternaria* spores at Cardiff during 1942 and part of 1943.

Visit to the National Museum of Wales

Miss E. A. JENKINS and H. A. HYDE. The collection of fungus models in the Department of Botany.

Meeting held at the London School of Hygiene and Tropical Medicine at 2.15 p.m., 16 November 1945. The President, Mr G. Smith, in the Chair.

R. A. WEBB. The microbiology of some African native beers.
G. SMITH. Introductory talk on an Exhibit of Moulds and Mould Products.

ANNUAL GENERAL MEETING, 1945

The forty-ninth Annual General Meeting was held in the Biology Department, Chelsea Polytechnic at 12 noon on Saturday, 8 December 1945, with the President, Mr G. Smith, in the Chair.

After the Minutes of the previous Annual Meeting had been read and signed the President recorded with deep regret the deaths of three members: Mr M. Barrowcliffe, Mr C. H. Grindling, and Professor H. H. Whetzel.

Reviewing the past year the President said: 'The outstanding events of 1945 have been the terminations of the two wars, with Germany and Japan, and although hostilities in Europe did not end till May, our programme was carried out as planned. Four ordinary meetings were held, one in this room, one at Kew, one at Cardiff, and a fourth at the London School of Hygiene and Tropical Medicine. In addition, there was a very successful two-day Phytopathological meeting at East Malling in July, a meeting held over from the time of the flying bombs of 1944. All who were present will remember the superb organization by the staff of the Research Station—and, possibly, that in 1945 apples ripened a month earlier than usual. The Foray programme was rather more ambitious than last year and included a series of forays on four successive days at Haslemere.

Last year my predecessor drew attention to a Report on the Need for Encouraging the Study of Systematic Mycology in England and Wales, which had been issued and widely circulated by the Council. As a sequel to that Report the Council has this year appointed a sub-committee to enquire into and report on the teaching of mycology in this country. The sub-committee has met several times under the chairmanship of Professor Ingold. A questionnaire was sent out to all heads of Departments of Botany and to members of the Society who are teachers. A large number of replies have been received and analysed, and a report is now in preparation.

Publication of the *Transactions* is still hampered by lack of paper. It is probable that next year's *Transactions* will have to be issued as double numbers but it is hoped that

normal quarterly publication will be resumed in 1947. Arrangements have been made by the Council for the preparation of a much needed supplement to the *Transactions* in the form of a collective index to the first fifty years.

The absence of two familiar names from the list of the Council's nominations for Officers for 1946 will no doubt have been noticed.

Dr Wormald has been an Editor of the *Transactions* for the last sixteen years, and it is with great regret that the Council has, at his request, withdrawn his name from the list of nominations. Dr Wormald is our greatest authority on diseases of fruit and hops, and his wide knowledge of plant pathology has been of the utmost benefit to the Society in his editorial capacity.

The other name which is missing, and yet not missing, from the nominations is that of Dr Ramsbottom, our President-Elect for 1946. Dr Ramsbottom has been General Secretary of the Society for twenty-five years, and in that capacity has given the most ungrudging service. We are fortunate in that his relinquishing the post of General Secretary means only that he will continue to serve the Society in another capacity.

The Treasurer, Mr A. A. Pearson, then submitted his statement and the accounts were adopted.

The following Officers and Members of the Council for 1946 were elected: *President*, J. Ramsbottom; *Vice-Presidents*, C. T. Ingold and C. Rea, in addition to the two past Presidents, G. Smith and R. W. Marsh; *Secretary*, G. C. Ainsworth; *Treasurer*, A. A. Pearson; *Editors*, B. Barnes and W. C. Moore; new *Members of the Council*, H. E. Croxall, S. J. Hughes, and F. A. Sawter (to replace Messrs E. A. Ellis, C. T. Ingold and J. H. Western). The Plant Pathology Committee's nominations of D. L. G. Davies, J. Grainger, and I. W. Prentice (to replace Messrs G. R. Bisby, M. H. Moore, and J. H. Western) were agreed to.

The President then moved from the Chair that Dr J. Ramsbottom be elected as an Honorary Member and this was agreed to with acclamation. Dr Ramsbottom briefly replied. Eleven new Members were elected making a total of thirty-two for the year.

After the 1946 programme had been discussed the meeting adjourned until 2 o'clock when the President delivered his address, 'Mycology and the War', after which Dr Barnes moved a vote of thanks to Mr Smith.

G. C. AINSWORTH, *Secretary*

RECEIPTS AND PAYMENTS

for the Year ending 30 June 1945

1944			£	s.	d.	1945			£	s.	d.	
1 July	Balance brought forward	...	526	3	11	2 June	Cambridge University Press: Cost of Vol. XXVIII <i>Transactions</i> , Reprints, and Common British Plant Diseases	527	4	0		
1945												
2 June	Sale of <i>Transactions</i> :											
	Current Volume	...	120	15	0							
	Back Volumes	...	81	18	4							
	Reprints	...	67	6	0							
30 June	Subscriptions to date	...	325	3	8	30 June	Postages, Stationery, etc.					
	Associates Subscriptions	...	1	0	0		Secretary	...	10	10	3	
	Gifts to Printing Fund	...	4	0	0		Sec. Path. Committee	...	3	5	0	
	Bank Interest	...	1	0	0		Editor	...	1	11	10	
	War Loan Interest	...	23	10	0		Treasurer	...	3	9	3	
							Sundry Printing	...	24	6	7	
							Fees of Meetings	...	3	1	0	
							Hire of Projector	...	1	10	0	
							Subscription to Council for Promotion of Field Studies			5	0	
							Balance at Bank	...	575	14	0	
			£1150	16	11					£1150	16	11

<i>CAPITAL ACCOUNT</i> —Robinson Bequest	£50	} invested in	£255 3	⁹ / ₁₆	War Stock.
Cheeseman Fund	£150		£245 3	⁹ / ₁₆	War Loan.
Printing Fund	£500		£200 3	⁹ / ₁₆	Defence Bonds.

A. A. PEARSON, Hon. Treasurer

Examined and found correct, E. W. SWANTON

FORAY REPORT, 1945

By the Foray Committee

(G. C. Ainsworth, R. W. G. Dennis, Miss M. P. English,
C. T. Ingold, Mrs E. W. Mason, *Secretary*)

A token Spring Foray was held on Saturday, 12 May, when the Clockcase Plantation, Englefield Green was visited by the Society for the first time in spring. Difficulties of transport and accommodation again made an Autumn Foray on a pre-war scale impossible and the main feature of the season was a series of four day-forays on 14-17 September in the Haslemere district with headquarters at the Haslemere Educational Museum, by kind permission of the Management Committee. Day forays in the London area were also held at Park wood, Ruislip (22 September), East Malling (29 September), Brickett wood (a joint foray with the Hertfordshire Natural History Society) (6 October), Epping Forest (including another visit to the Cuckoo Pits area with the Chingford Branch of the London Natural History Society) (13 October), and Bookham Common (a joint foray with the Ecological Section of the London Natural History Society) (28 October). A Cardiff foray planned for 21 October had to be cancelled because of torrential rain but one enthusiastic member spent some time collecting aquatic Phycomycetes in ponds at Dinas Powis. As in previous years, several natural history societies invited our members to attend their forays.

At the spring foray two areas in the Clockcase Plantation were sampled. In the first, sweet chestnut and birch predominated, and the second was a pure stand of Scots pine. Fine specimens of *Daldinia concentrica* were found on birch in the former area and it was noticed that most of the infected trees had suffered fire injury, possibly the result of a flying bomb which had fallen nearby during the previous summer. Another interesting find on the same day was a medlar tree in the botany garden of the Royal Holloway College showing severe leaf blotch caused by the conidial state of *Sclerotinia Mespili*, the affected tree scenting the whole garden.

Heavy rain severely limited the collecting on the first day of the Haslemere foray and on the other days the effects of the previous dry spell were still apparent. Among the 175 species of larger Basidiomycetes represented in the four days collections were *Inocybe globocystis* Vel. and *I. griseo-lilacina* Lange (which are both new records for Britain), *I. hystrix*, *Cortinarius delibutus*, *C. saniosus*, *C. balaustinus*, *C. damascenus*, *C. bolaris*, a small form of *C. evernius*, a small brown *Telamomium* tentatively identified as *C. penicillatus*, *C. melleopallens*, *Russula gracillina* (see Pearson, *Trans. Brit. mycol. Soc.* xxiii, 308), *Polyporus Schweinitzii* parasitic on cedar, *Sparassis laminosa* (a very large specimen at the foot of a living pine), *Hydnum scrobiculatum*, and *H. graveolens*.

Some interesting Hyphomycetes were also found at Haslemere; *Gonatobotryum fuscum* was found growing abundantly on oak wood cut for firing, and *Zygosporium parasiticum* was isolated from the same wood. The first is a new record for Britain; the second was originally described from Ireland and has been found once in Wales, but not previously in England. Other Hyphomycetes were: *Cordana pauciseptata*, apparently new for Britain, and *Botryosporium longibrachiatum* on dead tomatoes under glass. (The out-of-doors species, usually recorded as *B. pulchrum*, has this year been abundant elsewhere.)

Of Pyrenomycetes, *Ophionectria cerea* was found at Grays Wood again, this time associated with *Anthostoma turgidum* on beech; historically it has usually been recorded as associated with *Diatrype stigma*. *Hypoxylon fuscum* was found for the third time in twenty years, growing on dead birch; every year it is abundant on hazel, and common enough on alder. About a dozen specimens of *Cordyceps capitata* were collected on *Elaphomyces* in Grays Wood and *C. ophioglossoides* was also recorded.

Holt wood at East Malling was again visited and the sweet chestnut plantation was sampled. The general collection was much smaller than in 1944, *Geastrum fimbriatum* and *Clavaria Kunzei* being among the more interesting finds.

A large party worked Brickett wood on 6 October and although agarics were not very plentiful a good and interesting collection of microfungi was made; the day was notable

for the collection, by Mr N. F. Robertson, of perithecia of the oak mildew hitherto unknown in Britain.

The Epping foray gained a certain notoriety in the press from the presence of *Picture Post* photographers. The main party worked the forest between Loughton and High Beech where the collection was laid out and in the afternoon a few additional records were made in the Cuckoo Pits area. *Oidium aureum* was found on beech in the morning.

The foray to Bookham Common on 29 October though late in the season would have proved more fruitful had the weather been kinder. Even so a good display was set out in the waiting room at Bookham station and provisionally named before members moved on to Effingham Junction for tea. A number of very large specimens of *Lycoperdon saccatum* were collected and specimens of *Hygrophorus laevis* and *H. olivaceoalbus* were brought in.

The Foray Committee is greatly indebted to Mr Pearson, Mr Swanton, and Miss Wakefield for making critical determinations of agarics and for checking others, and to Mr E. W. Mason for the note on the Hyphomycetes found at Haslemere.

REVIEWS

Fungicides and their Action. By J. G. HORSFALL. (Waltham, Mass.: Chronica Botanica Co.; London: Wm. Dawson & Sons, 1945.) 239 pp. 24 figs. \$5.00.

'The writing of this book has constituted for the author a post-graduate course on fungicidal action', writes Dr Horsfall in the preface, and the reader will profit likewise, whether he is on the threshold of a research career or a more experienced scientist responsible for imparting knowledge and inspiration to others.

The book, with a foreword by Dr D. G. Fairchild, is the second of a series of separate memoirs—*Annales Cryptogamici et Phytopathologici*—devoted to general and systematic cryptogamy and to phytopathology', of which Mr S. D. Garrett's *Root Disease Fungi* was the first. Well documented, and with a bibliography of nearly 500 titles, it shows a confidently critical approach to the literature, which is fairly comprehensively reviewed. There is a general index and an author index.

The first of the sixteen chapters comprises an interesting and useful review of landmarks in the history of fungicides, beginning over two thousand years ago! The second is a necessary prelude to the main body of the work, for it defines the author's general conception of and approach to the problems dealt with in detail later in the book. Thus the reader is early prepared for the stimulation of Dr Horsfall's argument, though he may wish still to debate certain of the issues. Chs. III and IV concern laboratory bio-assay and the statistical treatment of data, subjects that largely provide the warp for the woof of later chapters. 'We are coming rapidly now to a stage where the vagaries of disease control can be studied more thoroughly and accurately in the laboratory than in the field' (p. 18)—conceivable, perhaps, of fungicidal action, but highly controversial of the much wider field of disease control. There is a short section on plant disease measurement in the field from the research angle (pp. 38–41) of special interest to plant pathologists. The principles of plant protection by chemicals are reviewed in Ch. V, and the next four chapters contain a comprehensive dissertation on the factors concerned in the deposition, coverage, and tenacity of fungicides. Recent theories of artificial immunization and chemotherapy receive attention in Ch. X, and the next four deal primarily with chemical problems—the action of copper, of sulphur, and of organic nitrogen and other organic compounds. A chapter on antagonism and synergism in fungicides precedes the concluding one, on Phytotoxicity—perhaps unwittingly a salutary reminder that the host plant may have the final word! Here it may be remarked that the author, by a seemingly unwarranted process of reasoning, arrives at the generalization that 'a fungicide is a chemical to kill plants' (p. 172).

Dr Horsfall adopts a racy, forthright, almost aggressive style throughout, which compels the reader's interest but may generate in some an 'equal and opposite' reaction. His very lively and critical probing of the many and diverse theories of others cannot but

impress, and the liberal use of homely analogy and exposition to enlighten some of the more knotty technical problems makes for easy reading and comprehension. A regrettable intrusion in a scientific work is the free use of parochial slang, most of which is necessarily transient and by many will not be understood.

This is a very useful and stimulating book at a reasonable price, and is a noteworthy addition to the literature because much up-to-date information has been skilfully drawn together from many different sources and arranged as a valuable work of reference. A few errors here and there (e.g. 'illusions', p. 62) do not unduly intrude.

M. H. MOORE

Flora Agaricina Danica. By JAKOB E. LANGE. 5 vols. (Society for the Advancement of Mycology. 23 Rolighedsvej, Copenhagen.)

Although we had heard with deep regret that Jakob Lange had died during the war, we have received with much satisfaction the fifth and last volume of his *magnum opus*. This completes a work written in English which no serious student of the agarics (if he or she can afford it) should be without. It should certainly be in the library of most natural history museums and universities. It is of value to the beginner and the expert. More than a thousand species are described and each one has a coloured figure (or portrait, as the author loved to call them) beautifully drawn and reproduced. The descriptions are brief because most specific details can be seen on the 'portraits' and the author even recommends that they should be examined with a lens. Microscopic features of diagnostic importance with accurate measurements are given, including spores, cystidia and sometimes the cellular structure of the cuticle of the pileus.

On the whole Lange was conservative in his classification. He adopts Quélet's genus *Rhodophyllus* for all the species with pink angular spores, but otherwise there are few departures from the Friesian groupings. Here and there he introduces a new genus, not always with happy results. His idea of some species may need correction, but many others which badly needed an authoritative interpretation will be accepted.

Lange wrote English with great fluency. The writer of this review looked through most of his manuscripts before publication and had many a lively discussion about the English idiom, which had its effect in the final script sent to the printers. The style, however, is his own and is clear and concise.

Flora Agaricina Danica followed the series of twelve brochures which Lange began in 1914 and finished in 1938, under the title 'Studies in the Agarics of Denmark'. They were published in the *Dansk Botanisk Arkiv*. The original object of these booklets was to provide accurate microscopic data for the agarics. The first, which dealt with *Mycena*, made the study of this genus comparatively easy. The others never quite reached the high standard set by the first, but they all had some features that were helpful especially the dichotomous keys to species. When Part 6 was reached dealing with *Russula* and *Psalliota* he found it necessary to do more than give microscopic data. In this and subsequent parts, he included short descriptions of each species. But these booklets were intended only to be used in conjunction with his water-colour drawings which were in the library of the Botanic Garden, Copenhagen, and therefore only available to the few. At last he was offered sufficient support to allow a wider public to take advantage of his admirable 'portraits'. The financial risk must have been considerable, but was sponsored by various Danish scientific societies and the first volume issued in 1935. When the war came, there was still one volume to appear. The author was spared to see it completed in 1941, and he died early in 1942.

Jakob Lange was well known as an authority on agriculture. He had been for many years the principal of the Small Holders' School. Mycology was a pastime; but one to which he brought all the keenness and powers of observation that had served him and others so well in his professional career. The occupation of his country by the German army was a hard blow. In the words of his son: 'he always loved freedom and hated nazism. In the last years of the occupation, his old home, the Small Holders' School, was taken over by the Gestapo and they made it the worst prison in the whole country. At last it was completely destroyed by the R.A.F.'

It was perhaps fortunate that he did not live to see this. A more enduring monument than the school is this work which he has bequeathed to future mycologists.

A. A. PEARSON

Die Hefesammlung des 'Centraalbureau voor Schimmelcultures'. Beiträge zu einer Monographie der Hefearten. II. Teil. Die anaskosporogenen Hefen. Zweite Hälfte. By H. A. DIDDENS and J. LODDER. (Amsterdam: N. V. Noord-Hollandsche Uitgevers Maatschappij, 1942) xii+511 pp., 99 figs.

The monographic survey of the yeasts, based on the investigation of the extensive collection at Baarn, and already well known to mycologists by the volumes issued in 1931 (N. M. Stelling-Dekker, *Die sporogenen Hefen*) and 1934 (J. Lodder, *Die anaskosporogenen Hefen*, Erste Hälfte), is continued in a third volume published in 1942, and now becoming generally available with the liberation of Holland.

This volume describes the three genera referred to the Mycotoruloideae, a subfamily of the Torulopsidaceae. It begins with an account of the characteristics and systematics of the subfamily, and of the materials and methods used by the authors. The main part of the book, occupying nearly 400 pages, contains an exhaustive description of the three genera (*Candida* with twenty-five species of which seven are new, and eight new varieties; *Brettanomyces* with four species; and *Trichosporon* with six species including two new species and two new varieties). A summary of the work, Latin diagnoses of the genera as amended by the authors and of the new species and varieties, with comprehensive indexes of authors and of systematic names, complete the work.

In the preparation of the book, 288 distinct stocks of *Candida*, seventeen of *Brettanomyces* and forty-three of *Trichosporon*, were used. Full details of the sources of these stocks are listed. The long lists of synonyms and the critical discussions of the opinions of other investigators of these fungi show that Diddens and Lodder have drawn upon every available source of information in forming their own conclusions. The great amount of research which has gone to the making of the book establishes the book as one of outstanding authority.

Many of the species described, especially of *Candida* and *Trichosporon* have been prominent for years past as pathogens of men and animals. To the ordinary mycologists, many of these species have been puzzles, the more so as the various authors who have concerned themselves with these fungi have often used methods more familiar perhaps to bacteriologists than to mycologists. Much of the earlier work does not fit into the traditional framework of mycology with its morphological bias, for these fungi are not well dowered with morphological characters on which separations can be made. Diddens and Lodder have now established the importance of physiological differences in determining the systematic status of the members of the Mycotoruloideae. They have done much to drive a path through a hitherto trackless waste of synonyms and of uncoordinated observations and to open up that forbidding subject often called medical mycology. It is on this account particularly that the book is very welcome. With this and the preceding monographs we can form a much more satisfying picture of the yeasts and it is to be hoped that we may have in due time an English version of the whole of the work. These monographs from Baarn should do much to attract more workers into a field of mycology where still there are harvests to gather.

Warm congratulations are due to the authors, and to the Centraalbureau voor Schimmelcultures, for finishing and issuing the volume at a time when Holland was occupied by the enemy and when there must have been many hindrances, material and spiritual, to the prosecution of work of the kind. It is to be hoped that the Centraalbureau will continue to use its collections as a means of providing surveys, not only of the yeasts, but of other groups of fungi to which clear guides are still needed.

B. BARNES

NOTICE

List of Common British Plant Diseases. A photographic reprint of the list is now available, price 5s. net (postage 3d.), and may be obtained from the Cambridge University Press.—Eds.

OOGENESIS AND FERTILIZATION IN *ISOACHLYA*
ANISOSPORA VAR. *INDICA*

By K. S. BHARGAVA

(With 2 Text-figures)

INTRODUCTION

Sexual reproduction in the Saprolegniaceae has been of interest for many years and for long it was a subject of controversy. Trow (1895) was the first to show that fertilization occurred in this family: subsequently, the occurrence of fertilization has been demonstrated in other genera of the family. For a review of the literature the reader is referred to Shanor (1937) and Wolf (1938).

The purpose of this study has been to follow the development of the sexual organs in living condition and to make a cytological study of fertilization in *Isoachlya anisospora* (de Bary) Coker var. *indica* Sak. & Bhar., a genus hitherto uninvestigated.

MATERIAL AND METHODS

The material was obtained from a local pond (Saksena & Bhargava, 1944). Cultures of the fungus were maintained on halves of hempseeds in distilled water. Sexual organs were produced in abundance when hempseed cultures were placed at a temperature of 20° C. Their development in living condition was studied under a water immersion lens. They were also killed and fixed in various stages of development using chromo-acetic acid solution, Claussen's chromo-acetic acid (Claussen, 1908) and Raper's chromic acid formalin solution (Raper, 1936). The last-named solution gave the best results. The time for fixation varied from twelve to twenty-four hours. After fixation, the material was washed, dehydrated, and embedded in paraffin in the usual manner. Sections were cut 4-5 μ thick and were stained on the slide with crystal violet according to Gram's technique described by Couch (1932) and modified by Shanor (1937).

OBSERVATIONS ON LIVING MATERIAL

The oogonia arise singly as terminal bodies on long slender branches of the hyphae. Sometimes intercalary oogonia are produced. The branches destined to form oogonia become densely filled with cytoplasm. The tip of the branch begins to swell by streaming in of the cytoplasm and this continues till it becomes rounded to form the young oogonium. The cytoplasm at this stage is more or less homogeneous and densely packed (Fig. 1 a).

Soon a central vacuole appears in the young oogonium (Fig. 1 b), which becomes cut off from the parent hypha by a transverse wall at its base,

leaving only a short neck (Fig. 1c). Before the transverse wall is laid down, several antheridial initials, arising androgynously or diclinously, come to touch the oogonium, and antheridia of various sizes are cut off by transverse walls. The cytoplasm which enters the antheridium is not very dense. After delimitation the antheridia elongate and encircle the oogonium in an ellipsoidal curve.

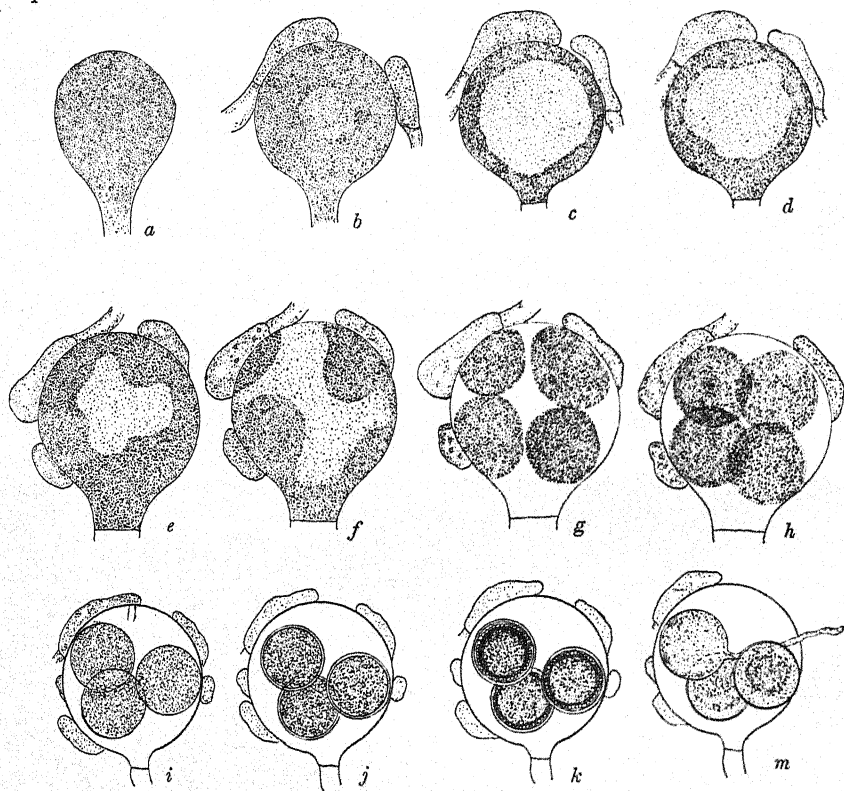


Fig. 1. Development of the oogonium, antheridium and oospore in living condition. $\times 1025$. *a*, swelling of the tip of a hypha forming oogonium initial. *b*, appearance of a small vacuole in the centre and two antheridia attached on the wall. *c*, appearance of a transverse wall at the base and enlargement of the vacuole. *d*, the vacuole producing cleavages. *e*, cleavages more prominent showing heapings of the cytoplasm. *f*, cleavages still more marked and cytoplasm in the form of roughly conical masses. *g*, oogonium initials being formed. *h*, oospheres maturing. *i*, a fertilization tube has made entrance in the oogonium. *j*, young oospores with their wall thickened. *k*, thick-walled mature oospores with oil droplets formed. *m*, an oospore germinating in an oogonium. Others are still dormant.

The central vacuole enlarges considerably, pushing the cytoplasm towards the periphery (Fig. 1c). It then sends out projections (Fig. 1d, e) which by furrowing the cytoplasm separate it into definite conical masses (Fig. 1f). The intervening cytoplasmic areas between these masses become thinner and thinner till they are ruptured by the extending cleavages. The separate masses of the cytoplasm swell up and begin to

round themselves to form oospheres (Fig. 1g), which, when fully grown, appear as perfectly spherical bodies (Fig. 1h). The number of oospheres ranges from one to ten; the oospheres measure $21.8-55.7\mu$ in diameter.

The coarse, granular appearance of the contents of the oospheres changes and oil globules begin to appear in the cytoplasm. Each oosphere has a thin membrane around it (Fig. 1i). Soon after this, fertilization tubes are sent into the oogonium by the antheridia but they are visible only for part of their length (Fig. 1i). The cytoplasm from the antheridia passes into these tubes but I could not see its migration into the oospheres.

Each oosphere now surrounds itself with a thin wall which gradually gets thicker and thicker till it becomes 3μ thick. This is the oospore (Fig. 1j). Equal sized oil globules are formed and arranged in two or three rows about the periphery of the oospore giving a characteristic centric or sub-centric arrangement (of oil droplets) (Fig. 1k).

During the process of oospore germination, which is rather rare and seems to be governed by some factors as yet not understood, the thick wall of the oospore gradually becomes thin. The oospore germinates *in situ*, after two days giving out a germ tube (Fig. 1m). The oil globules gradually disappear probably providing nourishment to the germinating oospore, in which a vacuole also appears. The germ tube elongates, comes out piercing the wall of the oogonium and gives rise to the mycelium. I did not observe the formation of sporangia containing spores on the short hyphae formed by the germination of oospores as observed by Trow (1899) in *Achlya americana* or by Weston (1918) in *Thraustotheca clavata*.

The antheridia remain attached to the oogonial wall until after the oospore germinates.

EFFECT OF TEMPERATURE ON THE PRODUCTION OF SEX ORGANS

To find out the optimum temperature for the production of sex organs, the fungus growing on hempseeds was kept at various temperatures ranging from 5 to 30° C. It was observed that at 5° C. only a few oogonia appeared, some of which did not have antheridia. Cultures kept at 10 and 15° C. produced an abundance of oogonia but not all of them bore antheridia. At 20° C. the fungus produced numerous oogonia, all of which bore antheridia. The temperature of 25° C. had a retarding effect on the formation of antheridia, since only a few of the oogonia formed had antheridia. The high temperature of 30° C. completely inhibited the formation of sexual reproductive bodies.

OBSERVATIONS ON FIXED MATERIAL

The young oogonium is filled with dense cytoplasm and contains many nuclei (Fig. 2a). The structure of the nuclei is similar to that of the nuclei in the vegetative hyphae. The nuclei are small, spherical, and contain a conspicuous central nucleolus from which a number of chromatin threads radiates.

In the next stage, a vacuole is seen in the centre of the cytoplasm, with

the nuclei shifted towards the periphery (Fig. 2*b*). The antheridium also is multinucleate. At about this stage the basal wall cutting off the oogonium from its parent hypha is complete.

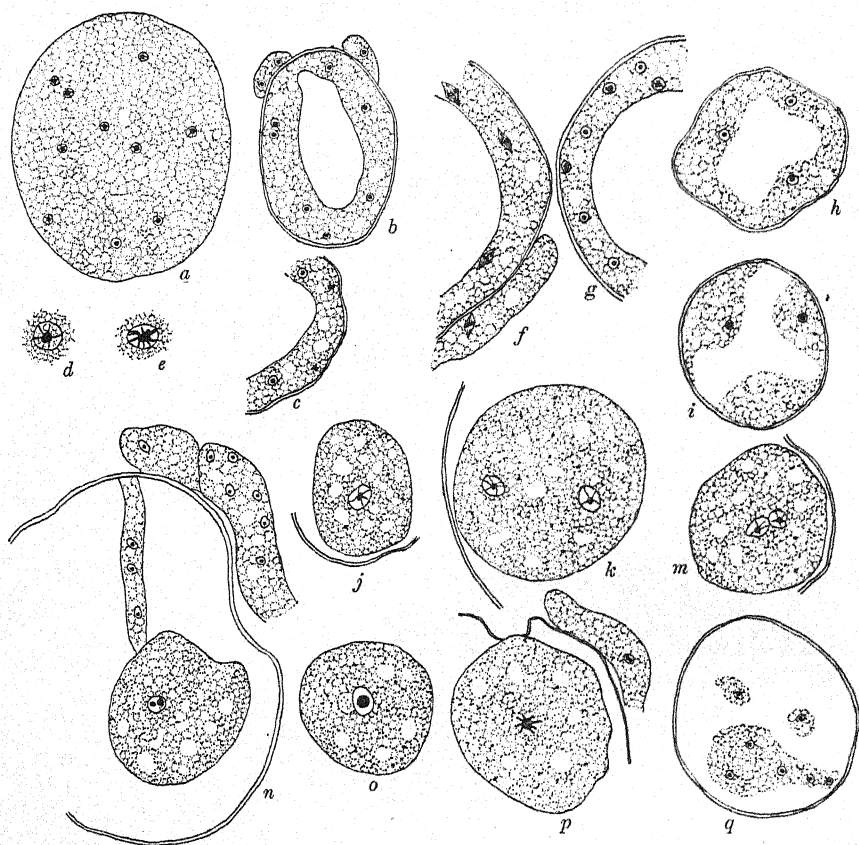


Fig. 2. Stages in the development of oospore in fixed material. $\times 1433$ except *d* and *e* which are $\times 3000$. *a*, section of a young oogonium showing nuclei in resting condition. *b*, section of an oogonium showing a small central vacuole and peripheral arrangement of the cytoplasm and the nuclei. Antheridia seen attached to the oogonium. *c*, section of an oogonium showing some nuclei in the process of degeneration. *d*, early prophase nucleus from an oogonium. *e*, late prophase nucleus showing the clustering of the chromatin into curious rod-like bodies. *f*, section of an oogonium and attached antheridia showing nuclei in metaphase. *g*, some of the daughter nuclei degenerating. *h*, section of an oogonium showing early stages in the cleavage of uninucleate egg origins. *i*, a later stage during the cleavage showing three separate oosphere initials. *j*, young oospheres with a single nucleus. *k*, an oosphere containing a female gamete nucleus in the centre and the male gamete nucleus towards the periphery. *m*, an oosphere showing male and female nuclei in contact. *n*, section of an oogonium showing multinucleate fertilization tube attached to an oosphere containing a fusion nucleus: two nucleoli are visible. *o*, a mature oospore containing a large fusion nucleus. *p*, a germinating oospore showing the nucleus in late prophase. Antheridia are still attached. *q*, a germinating oospore with a germ tube. Several nuclei are visible.

On account of the enlargement of the vacuole in the centre of the oogonium, the nuclei and the dense cytoplasm become limited to a

rather thin peripheral region. At a later stage, a number of nuclei are seen to be degenerating (Fig. 2*c*). The remaining nuclei then begin to divide. The oogonial nuclei enlarge considerably in early prophase (Fig. 2*d*). In late prophase the chromatin material becomes clustered around the nucleolus in the form of rod-like bodies (Fig. 2*e*). In the metaphase, nuclei show definite spindles, the longitudinal axes of which are nearly parallel to the wall of the oogonium. Some of the spindles may be seen to be surrounded by a nuclear membrane and are therefore intranuclear (Fig. 2*f*). Not all the stages from the prophase to the metaphase could be found. The chromosomes stain very darkly with gentian violet and are extremely small. They could not be counted with certainty but they are not less than eight. Simultaneously, the antheridia attached to the oogonium show nuclear divisions.

Following mitosis, most of the daughter nuclei degenerate, leaving only those which are to function as female nuclei (Fig. 2*g*). Eventually the contents of the oogonium are seen to be dividing up as the furrows advance from the vacuole (Fig. 2*h*). Each separate mass is uninucleate (Fig. 2*i*).

In the next stage, round oospheres lie within the oogonium and each has one nucleus in its centre (Fig. 2*j*).

The male gametic nucleus discharged from the fertilization tube lies near the periphery of the oosphere (Fig. 2*k*). It then moves towards the female gamete nucleus and comes to lie by its side (Fig. 2*m*).

In Fig. 2*n* a multinucleate fertilization tube from an antheridium is seen lying in contact with the oosphere, in the centre of which the male and the female nuclei are fusing. The membrane at the point of contact has been lost, and the fusion nucleus contains two nucleoli.

Finally the oospore is seen containing one nucleus which has enlarged considerably (Fig. 2*o*). The oospore nucleus, on germination, begins to divide. Fig. 2*p* shows the oospore nucleus in the late prophase. In the germinating oospore a germ tube is seen containing several nuclei (Fig. 2*q*).

The astral rays so prominently shown in *Leptolegnia caudata* by Couch (1932) and in *Thraustotheca clavata* by Shanor (1937), among other workers, were not seen in any stages of the development of sex organs.

DISCUSSION

The results of the investigation are in general agreement with those of other workers who have studied the development of sexual organs in the Saprolegniaceae, and definitely establish the occurrence of fertilization in *Isoachlya anisospora* var. *indica*. *Isoachlya* is, therefore, another genus added to the list of Saprolegniaceae in which fertilization has been observed.

As regards the nuclear division in the oogonium and antheridium, Shanor (1937), in *Thraustotheca clavata*, found that mitosis in the antheridium begins after the division of the nucleus has progressed in the oogonium. Wolf (1938) reported that the division of the antheridial nucleus precedes that within the oogonium. The results obtained here are contrary to those of Shanor (1937) and Wolf (1938) but they agree with the findings of other investigators (Kasanowsky, 1911; Patterson, 1927, etc.) who have

reported that the nuclear division in the oogonium and antheridium occurs simultaneously.

There is only one nuclear division in the oogonium and antheridium of *Isoachlya anisospora* var. *indica*. Trow (1904) claimed that two nuclear divisions occur within the oogonium of *Achlya de Baryana* and probably also in *A. polyandra*. His view has been disputed by all other investigators who have shown that there is only one nuclear division within the oogonium or antheridium.

The chromosomes in the nuclei of water moulds are extremely small and cluster together so closely that it is very difficult, if not impossible, to make an exact count. Up to this time all workers have been able to give the approximate number in the species investigated by them, excepting Trow (1895) who reports the presence of one chromosome in *Saprolegnia dioica* and *S. mixta*.

Most of the workers have reported the presence of a centrosome and astral rays radiating from it. Patterson (1927), however, did not observe these rays in *Achlya colorata*. Raper (1936) reported the absence of astral radiations in *A. bisexualis* and Wolf (1938) failed to observe centrosomes and astral radiations in *A. flagellata*, in which they had been observed by Moreau and Moreau (1935). Höhnk (1935) reported the presence of astral radiations but no centrosome in *Saprolegnia ferax*. I, like Wolf, incline to the view that their absence is perhaps due to the difference in the methods of fixation and staining employed.

SUMMARY

The general development and the cytology of the sexual organs of *Isoachlya anisospora* (de Bary) Coker var. *indica* is essentially the same as that of other members of the family Saprolegniaceae.

There is only one nuclear division in the oogonium and antheridium and it occurs simultaneously in both. The decrease in the number of nuclei is due to degeneration. No astral rays have been seen radiating from the nuclei. The number of chromosomes is not less than eight.

Fertilization has been shown to take place in *Isoachlya anisospora* var. *indica*.

I wish to express my warmest gratitude to Dr R. K. Saksena, University of Allahabad, for his advice and criticism throughout the course of the present study. My grateful thanks are here offered to Prof. S. R. Bose, Calcutta; Dr G. Watts Padwick and Dr B. B. Mundkur, New Delhi, for the generous loan of relevant literature and to Mr S. D. Garrett, Rothamsted Experimental Station, Harpenden, for kindly going through the manuscript.

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SIZE AND FORM IN AGARICS

By C. T. INGOLD, *Birkbeck College, University of London*

(With 3 Text-figures)

INTRODUCTION

On reading the second edition of D'Arcy Thompson's *Growth and Form*, it occurred to me that the concept of form tending to alter as a consequence of change of size, although it has been applied successfully to animals and green plants, has not been used in Fungi. Further it seemed that the agaric sporophore, because of its rather regular and geometric form, offered a good subject for testing the general thesis that form tends to vary with size, especially as the range is so great in Agaricaceae with *Marasmius Hudsonii* (pileus $\frac{1}{4}$ in. diam. \times sporophore 2 in. high \times stipe $\frac{1}{64}$ in. thick) at one extreme and *Psalliota villatica* ($14 \times 7\frac{1}{2} \times 3$ in.) at the other.

In the fruit-body of an agaric of the usual form the central stipe has, as Buller (1909) pointed out, two chief functions: the support of the cap, and the provision of a space below the pileus where air currents may have a reasonable opportunity of wafting the spores away before they reach the ground in the immediate vicinity of the sporophore.

CAP DIAMETER AND WIDTH OF STIPE

The weight of the cap in an agaric may be taken as roughly proportional to its volume. This weight acts vertically on the stipe and the strain is distributed over the area of the cross-section. If it is assumed that organisms are economical with their tissues, it might be expected that the stipe would tend to be of a thickness just sufficient to support the pileus with the rigidity necessary for the proper functioning of the sporophore.

In solid figures which vary in size but not in form, the volume varies as the cube of the linear dimensions (e.g. height or breadth), but the surface, and other two-dimensional features, vary as the square of these dimensions. Thus the volume, and, therefore, the weight, of the pileus varies as the cube of its diameter, but the cross-section of the stipe varies as the square of its width. These considerations would lead to the expectation that the sporophore would not remain constant in form with change of size but that, as compared with a central type, the smaller fruit-bodies would tend to have relatively slender stipes and the larger ones relatively thick ones.

To test this expectation the agarics listed in Ramsbottom's *Handbook of the Larger British Fungi* have been considered, omitting only species in which the stipe is lateral or wanting. The dimensions of the fruit-bodies, derived from an earlier handbook by W. G. Smith (1908), are given in the following form: e.g. *Amanita phalloides* $3\frac{1}{2} \times 4\frac{1}{2} \times \frac{5}{8}$. Smith remarks: 'The dimensions give the typical size in inches of well-developed examples as

found in Britain. . . . The numerals represent the diameter of the pileus, the height from the base of the stem to the top of the pileus, and the diameter of the stem near the middle.' The figures are clearly estimates and not objective determinations based on actual measurements of a large number of specimens of each species. It seems probable, however, that dealing with such large numbers (580 species) imperfections of the data may largely average out and the general pictures be not obscured.

In Fig. 1, with diameters of the pileus as abscissae and diameters of the stipe as ordinates, most of the species of Ramsbottom's *Handbook* are plotted, except for thirty very large ones which fall outside the area of the figure.

The average cap diameter for all the 580 species is 3.065 in. and the average stipe diameter 0.505 in. To make the arithmetic easier, a fruit-body having a cap 3 in. across and a stipe $\frac{1}{2}$ in. thick has been taken as the average type. The straight line in Fig. 1, drawn through the origin and through the point representing this 'average type', would be the graph of cap width plotted against stipe thickness if the *form* of the 'average type' were maintained irrespective of size. If the actual values plotted in Fig. 1 tended on the average to conform to this graph, they would be distributed

Table 1

Diameter of stipe (in.)	No. of species	Average pileus width
$\frac{1}{16}$	26	0.96
$\frac{1}{8}$	63	1.25
$\frac{3}{16}$	35	1.94
$\frac{1}{4}$	89	2.15
$\frac{3}{8}$	58	2.63
$\frac{1}{2}$	91	3.33
$\frac{3}{4}$	78	3.77
1	66	4.55

equally to left and right of this line throughout its length. This is not so. In the lower region the points are mainly to the right of the line and in the upper region to the left. It is clear that with changing size the *form* tends to alter and the smaller fruit-bodies have relatively slender stalks whilst the larger ones have relatively thick stalks.

In Ramsbottom's *Handbook* certain values for thickness of stipe are very commonly recorded. These are given in Table 1 together with the average pileus width for all those species listed as having each stipe diameter.

The values are also plotted in Fig. 2. In the same figure the straight line $y = 0.16x$ is shown, y being the diameter of the stipe and x that of the pileus. It is the same line as that drawn in Fig. 1. The equation is derived from the dimensions of the 'average type' where $y = 0.5$ and $x = 3.0$. The curve in Fig. 2 corresponds to the equation $y = \sqrt{(0.0092x^3)}$, and is the curve which might be expected if, as the volume (weight) of the cap varies, the diameter of the stipe is so adjusted that the weight acting on unit cross-section remains the same. This curve also is derived from the 'average type' thus:

$$\frac{y^2}{x^3} = \frac{0.5^2}{3.0^3} \quad \text{or} \quad y = \sqrt{(0.0092x^3)}.$$

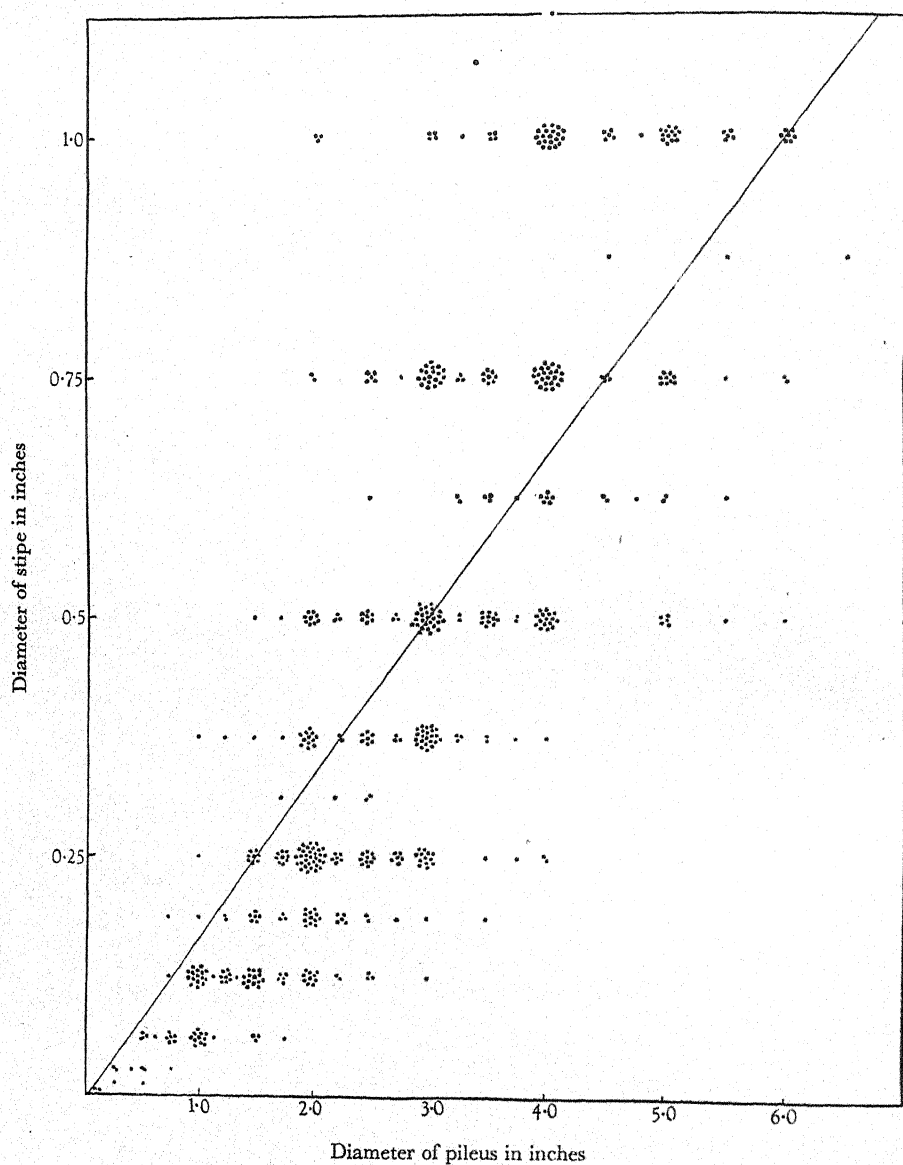


Fig. 1. Diameter of cap plotted against diameter of stipe. Each dot represents a single species. Where two or more points should be in the same position, they have been grouped close together around this position.

Clearly this equation and $y = 0.16x$ have two solutions in common, namely, $x = 0, y = 0$ and $x = 3.0, y = 0.5$.

An examination of Fig. 2 shows that the actual points do not conform with the straight line, but show quite a striking approximation to the curve.

It seems, therefore, that size and form are interrelated in agarics as in other organisms. Perhaps if a substantial body of objective data were

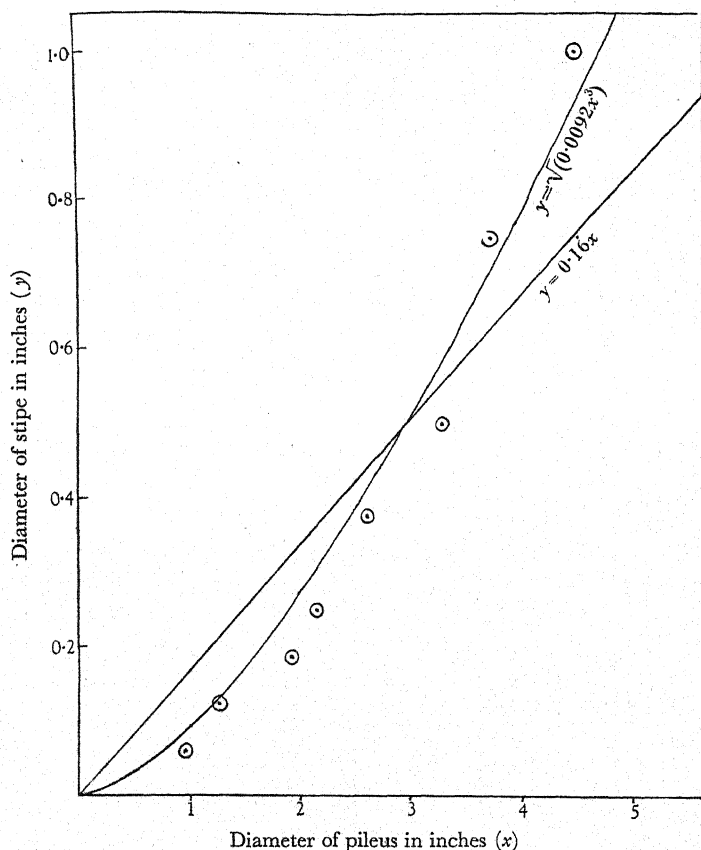


Fig. 2. For explanation see text.

available the relationship of size and form might be even more clearly demonstrable. In this connexion it would be most interesting to collect for a large number of species, and for a large number of individuals of each species, data of pileus weight and the corresponding area of cross-section of the stipe.

CAP DIAMETER AND HEIGHT OF SPOROPHORE

The height of an agaric is mainly due to the length of the stipe which provides a space between the underside of the pileus and the ground so that

spores in their downward course have a good chance of being blown away before they reach the ground in the immediate vicinity of the fruit-body. With the development of larger and smaller types of agarics it might be expected that the general form would tend to be preserved. However, in the larger types the interval between cap and substratum would become greater than necessary and it might be expected that mutants having shorter stalks, and thereby economizing tissue, would tend to survive in the struggle for existence. On the other hand, in the smaller types, if the form remained unaltered, the interval between the pileus and the ground might be reduced too far for efficient dispersal and mutant types with longer stalks would tend to be selected. Thus we might expect that the larger

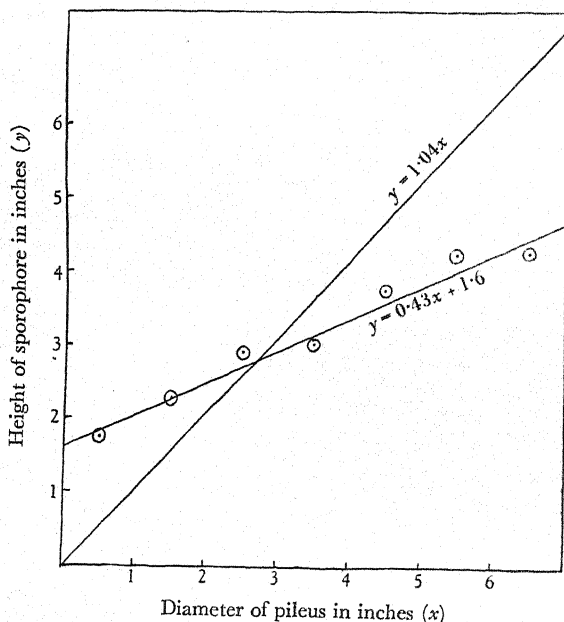


Fig. 3. For explanation see text.

sporophores would tend to be relatively short and the smaller ones relatively tall.

In Table 2 the species are divided up into size groups based on pileus width. The number in each group is shown with the average height for members of that group. These averages are plotted in Fig. 3 together with a straight line drawn through the origin and through the point representing the 'average type' (pileus diameter 3 in., height 3.1 in.). This line ($y = 1.04x$) would represent variation of height with pileus diameter if the form remained unaltered irrespective of size. Clearly the points depart greatly from this line. Indeed, they appear to approximate to a straight line of the type $y = mx + c$ and that drawn in Fig. 3 is $y = 0.43x + 1.6$. The value (0.43) of the constant 'm' is quite arbitrary and is selected merely to

Table 2

Pileus diameter Size group (in.)	No. of species in group	Average height (in.)
0-<1	22	1.75
1-<2	101	2.26
2-<3	147	2.89
3-<4	140	3.01
4-<5	85	3.73
5-<6	40	4.21
6-<7	15	4.25

give as close a fit as possible with the plotted points. The constant 'c', which appears to have a value of about 1.6 in., may be pictured as the average minimum height of fruit-body for efficient spore dispersal.

SUMMARY

Small agarics tend to be relatively tall and have relatively thin stalks, and large agarics tend to be relatively short and have relatively thick stalks as compared with an 'average type'. It is suggested that these tendencies for form to alter with size follow from simple mechanical and functional considerations.

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A STUDY OF VIOLET ROOT ROT

FACTORS AFFECTING PRODUCTION AND GROWTH OF MYCELIAL STRANDS IN *HELICOBASIDIUM PURPUREUM* PAT.

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(With Plates V and VI)

The symptoms of Violet Root Rot are so pronounced, and so obviously attributable to infection by a root-infecting fungus, that it would be difficult to mistake this disease for any other. It was therefore possible for Duggar (1915), in his review of early work on violet root rot, to cite an unmistakable description of this disease on saffron (*Crocus sativus*) published by Duhamel (1728). The causal fungus was named *Sclerotium Crocorum* by Persoon (1801), and was later transferred by De Candolle (1815) to his new genus, *Rhizoctonia*, which included two species, *R. Crocorum* on crocus and other hosts, and *R. Medicaginis* on lucerne. Later, after a thorough morphological study of violet root rot in a number of hosts, the brothers L. and C. Tulasne (1851) reduced De Candolle's two species, *R. Crocorum* and *R. Medicaginis*, to a single species, which they named *R. violacea*. Duggar (1915) confirmed the Tulasnes's conclusions, but pointed out that their new specific epithet *violacea* contravened the Rules of Nomenclature, and that the correct name was *R. Crocorum* (Pers.) DC. As Buddin and Wakefield (1924) comment: 'It is unfortunate that the name is less descriptive than *R. violacea*, and is particularly inappropriate in this country where the crocus disease is unknown.' It only remains to add that as, according to the International Rules of Botanical Nomenclature, fungus names date from the publication by Fries (1823) of the *Systema Mycologicum*, the correct citation is now *R. Crocorum* Fr. (British Mycological Society, 1944).

More than a century of search for the perfect stage of *R. Crocorum* was ended by its discovery in a Basidiomycete, *Helicobasidium purpureum* Pat., by Buddin and Wakefield (1927). They found fructifications of *H. purpureum* in very close association with violet root rot in a number of localities and on three different hosts, red clover, dog's mercury (*Mercurialis perennis*), and nettle (*Urtica dioica*). Morphological characters of the mycelium of *Helicobasidium purpureum* appeared identical with those of *Rhizoctonia Crocorum* both on the host and in pure culture, and conidia of the type *Tuberculina* were commonly produced in cultures of both forms. Finally, typical violet root rot was produced by inoculation with mycelial cultures derived from three distinct basidiospore isolates of *Helicobasidium purpureum*. Additional evidence in support of the connexion between *Helicobasidium purpureum* and *Rhizoctonia Crocorum* was published by Buddin and Wakefield (1929) two years later.

During the course of the investigations to be reported here, still further evidence in confirmation of Buddin and Wakefield's conclusion was incidentally obtained. In mid-April 1945, fertile fructifications of *Helicobasidium purpureum* on old cut stems of the previous season were found in boxes of red clover inoculated with a culture of *Rhizoctonia Crocorum* (originally isolated by Dr R. Hull from a sugar beet in Lincolnshire) in March 1944. The identification of *Helicobasidium purpureum* was confirmed by Miss Wakefield.

In this country at the present time, violet root rot is of economic importance chiefly as a disease of sugar beet (Hull & Wilson, 1946), though it also affects other field crops, such as clover and other legumes, mangold, carrot and potato, and a variety of more strictly horticultural crops, such as asparagus and seakale (Moore, 1943). The need for further study of factors influencing infection by the violet root rot fungus was emphasized by Buddin and Wakefield (1927) in their statement: 'It is obvious that one of the most pressing needs is for more exact information as to the conditions under which *Rhizoctonia* will infect living roots.'

DEVELOPMENT OF THE FUNGUS ON THE HOST

In plants inoculated in the glasshouse by means of sclerotia buried in the potting soil, a preliminary to infection can be noted three or four weeks after inoculation, in the form of red epiphytic mycelial strands attached at occasional points to the roots. After some eight weeks, infection cushions can usually be found. Prillieux (1891) was the first to point out that these infection cushions, or 'corps miliaries' as he called them, constituted the chief mode of tissue invasion by the fungus. Prillieux's view that these bodies were simply infection cushions was upheld by Duggar (1915) and Faris (1921). The infection cushions are connected at their apices with the mycelial web extending over the host epidermis, and are at least partially sunk into the cortical tissue; their development on young potato stems and tubers has been described by Faris (1921). It is unfortunate that, on account of their morphological resemblance to small sclerotia, these bodies have been termed microsclerotia by some writers: functionally, they are very different from the true sclerotia, which are produced towards the end of parasitic activity, and constitute the resting stage of the fungus.

The true sclerotium was first described by Duhamel (1728) as a 'tubercule', which he thought to be the fructification of a fungus related to the truffles. The sclerotia vary greatly in size, though usually not exceeding 5 mm. in length on the host plant; in form they tend to be elongated, and often with a suggestion of compound structure. Sclerotia may be produced on the mycelial web or strands, as described by Duggar (1915) and Faris (1921), but on sugar beet and clover they are most frequently formed around small lateral roots, along which they may even be strung like beads on a thread. The vascular elements of the rootlet can then be distinguished when examining a section of the sclerotium under the microscope. During the present investigation, isolations of *Helicobasidium purpureum* have been obtained without difficulty by surface sterilizing sclerotia for approximately

a minute in 1 : 1000 mercuric chloride in 10 % alcohol, washing in sterile water, and plating out on malt-extract agar.

In plate cultures of the fungus on a suitable agar, such as meat-malt extract agar of twice normal concentration, sclerotia begin to be discernible some four weeks (at 25° C.) after inoculation of the plates, and they continue to develop visibly for at least another month. A conspicuous feature of these sclerotia is their persistent golden-brown mantle of setae, which can be seen in Plate Xb of the paper by Buddin and Wakefield (1924). Sclerotia formed in plate culture are very variable in size, and compound sclerotia are common, especially around the site of the inoculum piece.

METHODS

Handling of soils and soil containers. Stocks of soil for experimental work are passed through a $\frac{1}{4}$ in. sieve, then air-dried and passed through a fine sieve (14-mesh per in.). The clods of soil are crumbled by hand, but not mechanically crushed, because heavy crushing breaks down some of the soil crumbs into their constituent particles, and so gives an abnormally fine tilth (Russell, 1938). After thorough mixing of the whole stock of air-dried soil, saturation capacity is determined by the perforated box method (Keen & Raczowski, 1921). For work with moisture contents down to 40 % saturation, the calculated amount of water can be added from a measuring cylinder to the surface of the air-dry soil in its individual container. For moisture contents of 30 % saturation or less, it is advisable to mix soil and water by hand before filling the containers, but after this procedure it is difficult to get as close or as uniform a packing of the moist soil as when water is added to the air-dry soil already settled in the container. For this reason, a moisture content of 40 % saturation was the lowest studied in the present investigation. A range of soils varying in texture can be obtained by progressive dilution of the soil with sand. A range of pH values is achieved by treating portions of soil with suitable amounts of sulphuric acid or calcium hydroxide. After treatment, the moist soils are incubated in 12 in. unglazed pots in the glasshouse for at least a month, for equilibrium with the reagents to be reached as far as possible; the treated soils are then air-dried for use in the laboratory. Wherever possible, factorial designs have been used for these experiments, and soil containers have been arranged in randomized blocks on the laboratory table.

Measuring growth of mycelial strands. The activity of root-infecting fungi on the host roots and in the soil has been investigated by the glass-tumbler method in two ways: (i) seedling plants have been used by Garrett (1936) for *Ophiobolus graminis*, by Shen (1940) for *Fusarium culmorum*, and by Samuel and Garrett (1945) for *Plasmodiophora Brassicae*; (ii) Rossi-Cholodny slides have been used by Blair (1943) for *Rhizoctonia Solani*. For the present investigation on *Helicobasidium purpureum*, Rossi-Cholodny slides were found satisfactory, but seedlings proved unsuitable; mycelial growth of *Helicobasidium purpureum* is slower than that of *Ophiobolus graminis*, and even four weeks after sowing and inoculating seed, the epiphytic mycelial

strands of *Helicobasidium purpureum* are still only lightly attached at occasional points to the seedling tap-root. A satisfactory alternative to seedlings was eventually found in a plant-storage organ, the potato tuber, and this was used in preference to the Rossi-Cholodny slide for the majority of experiments.

The potato-tuber method, as finally adopted, was as follows. The fungus was grown on a suitable agar medium, and when colonies were at an optimum age for production of inoculum, disks were cut all round, equidistant from the centre, with an 8.5 mm. cork borer. With the same cork borer, an approximately hemispherical piece of tissue was removed from the centre of the most even and regular face of the tuber, and the agar culture disk placed in the wound. The soil container, a 2 lb. glass jam jar, of diameter 8 cm. and height 14 cm., received first one-third of the total weight of dry soil, which was shaken down and then brought to the calculated moisture content. The tuber was placed, inoculum downwards, on the moist soil, and the remaining two-thirds of dry soil was added, shaken down and brought to correct moisture content. When different soils were compared, weights of soil were so adjusted as to give equal volumes; the moist soils, with tubers, filled the jars to a height of 8-9 cm. The experiments were allowed to run for some seven weeks in the laboratory (mean temperature 15-20° C.), soil moisture content being made up weekly on a box balance. Tubers were then washed out of the jars as gently as possible, so as not to dislodge the mycelial strands, and preserved in formalin for recording.

Growth of mycelial strands from the inoculum disk was measured under the binocular dissecting microscope, with magnification 20×; a dozen headed pins were used as combined dissecting needles and markers. For some distance out from the inoculum disk, the growth of the red mycelial strands could be followed with ease, sometimes even by the naked eye (Pl. V, fig. 1), but further out the strands became progressively thinner and paler in colour, so that a dissecting needle was used to tease them up from the tuber surface for better visibility. When the tip of the strand had been determined, its position was marked by sticking the needle into the tuber. When the tips of all the longer strands had been marked, the distance of the farthest from the inoculum disk was measured over the curved surface of the tuber by means of a paper scale marked in millimetres, and recorded as *maximum growth of mycelial strands*, for each tuber.

The type of mycelial disk inoculum was found to be of great importance, but its specification is best reserved for the next section. Wounding with the cork borer was subsequently found not to be necessary for production and growth of mycelial strands over the surface of the tuber, but was continued in order to protect the inoculum disk from possible desiccation immediately after covering the inoculated tuber with dry soil, when filling up the jar. Tubers of the potato varieties King Edward or Majestic were used according to convenience in all experiments; in a preliminary trial, there was no significant difference in extent of mycelial strand growth over tubers of these two varieties. Relatively young tubers were best for these experiments, owing to their lesser liability to bacterial rotting, especially in

soils of high moisture content, and their slower rate of sprouting in the jars. The sprouting of older tubers was not a serious disadvantage for the limited duration of these tests, however, because the incipient leaves on the sprouts failed to develop under laboratory conditions. A third disadvantage of old tubers, and one greater than either of these, was that resistance of the tuber to infection appeared to increase with storage age, as shown by a survey of all the experimental data. With increasing storage age of the tuber, from mid-August soon after digging (the earliest tested) to mid-April of the following year (the latest tested), distance grown by mycelial strands and frequency of infection cushions (Pl. V, fig. 2) both declined. Development of a progressive rot, in tubers infected by *Helicobasidium purpureum* while still attached to the parent plant, has been described by Faris (1921); an example of such progressive infection is illustrated in Pl. VI, fig. 1, showing two tubers collected from an infected potato crop. In Fig. 2 of Pl. VI is shown, for comparison, another potato tuber, also collected from an infected field, on which epiphytic strands of *Helicobasidium purpureum* are well developed, but unaccompanied by infection cushions. Of interest in this connexion is a statement made by a Northamptonshire farmer that he had clamped some potatoes which were covered with a brownish web of the violet root rot fungus, but were otherwise sound, and that when he opened the clamp in the following March, the potatoes, though still bearing the mycelium of the fungus, had not rotted.

EXPERIMENTS WITH POTATO TUBERS

The experiments with tubers are concerned first with the effect of type of inoculum upon production and growth of mycelial strands, and secondly with the effect of soil conditions upon such growth.

Experiment I. In a preliminary experiment, wounded potato tubers had been inoculated with mycelial agar disks cut from the growing margin of the fungus colony, but growth of strands was either poor and irregular, or lacking altogether. It was therefore decided to test marginal disks against submarginal disks taken from the same colonies; the submarginal disks were cut with the same cork borer immediately inside the ring of marginal disks. The different agar media employed were all variations of the meat-malt extract agar found by Buddin and Wakefield (1924) to be the best of any that they tested for growth of this fungus. The standard formula taken for this agar was as follows: Lemco beef extract 10 g., malt extract 30 g., agar 20 g., and distilled water 1 l. Four concentrations—nil, normal, twice and thrice normal—of each constituent, meat and malt, were tested in all possible combinations with the four concentrations of the other constituent (except that the 0 meat-0 malt combination was omitted). Variation in growth rate of the fungus on these different meat-malt agars, as shown by mean colony diameter of triplicate plates after twenty-four days at 25° C., is shown in Table 1.

Most rapid growth of *Helicobasidium purpureum* was made on 1 meat-1 malt agar, i.e. that prepared according to the standard formula, and growth fell off somewhat with higher concentrations of meat and malt.

After twenty-six days' growth of the colonies at 25° C., five marginal and five submarginal inoculum disks were cut from a plate of each type of agar, and inoculated into potato tubers, variety King Edward, which were then buried in jars of a mixture of 1 vol. Harpenden allotment soil : 3 vol. sand, brought to a moisture content of 60 % saturation. The experiment was set up on 8 February 1945, and was harvested after sixty days (mean laboratory temperature 20° C.): the results are given in Table 2.

Table 1. *Mean diameter of Helicobasidium purpureum colonies in mm.*

		Conc. of malt in agar			
		0	1	2	3
Conc. of meat in agar {	0	—	41	51	51
	1	28	59	57	52
	2	33	55	54	52
	3	30	45	46	45

Table 2. *Maximum growth of mycelial strands in mm.*

		Marginal disks Conc. of malt in agar				Submarginal disks Conc. of malt in agar			
		0	1	2	3	0	1	2	3
Conc. of meat in agar {	0	—	0	3	7	—	21	23	27
	1	0	1	8	7	0	5	22	33
	2	0	5	8	8	0	10	24	27
	3	0	11	12	15	0	19	36	29

The most striking feature of these results is the greater extent of strand growth from the submarginal disks. Also noteworthy is the complete absence of strand growth from disks of all the 0-malt agars. With a single exception, 3 meat-3 malt agar, strand growth increased with increasing concentration of malt in the agar. Optimal concentrations of meat and malt for production of strands were, however, supra-optimal for colony growth on the original agar plates (Table 1).

Experiment II. In this experiment, an explanation was sought for the better production and growth of mycelial strands from submarginal than from marginal inoculum disks. It seemed likely that in the marginal disks a higher proportion of the nutrients in the agar would still be outside the hyphae, and therefore available for assimilation by other soil micro-organisms in competition with *Helicobasidium purpureum*, after inoculation of the tubers. To test this possibility, marginal inoculum disks were cut from colonies on 2 meat-2 malt agar, three weeks old at 25° C., and treated in four different ways:

- A. Used at once for inoculation of tubers.
 - B. Incubated in moist sterile Petri dishes at 25° C. for one week.
 - C. Contaminated with soil suspension, and incubated in moist Petri dishes at 25° C. for one week.
 - D. Cut, but left in position in original agar plates for one week at 25° C.
- Ten potato tubers, variety Majestic, were inoculated with disks from each series, and buried in the 1 soil : 3 sand mixture, adjusted to a moisture

content of 60% saturation. Tubers were inoculated with disks of series A on 7 August 1945 and with those of the other three series one week later. Maximum strand growth was measured after seven weeks (mean laboratory temperature 17° C.) in all series, and results are given in Table 3.

Table 3. *Maximum growth of mycelial strands in mm.*

A. Disks used at once	28 ± 2.0
B. Disks incubated sterile	27 ± 3.4
C. Disks incubated unsterile	21 ± 2.8
D. Disks cut, but left in position	38 ± 2.3

It is evident that incubation of the marginal inoculum disks under moist sterile conditions for one week has not made them any more effective as inoculum than the same disks used immediately for inoculation of the tubers. Assimilation by other soil micro-organisms of the unconsumed nutrients in the marginal disks during the first week after inoculation cannot therefore be held responsible for the poorer strand growth from such disks than from submarginal disks. Although growth from the disks of series C, incubated unsterile for a week, was slightly less than that from disks of series A and B, this reduction has not reached the 5% level of significance (by the *t* test). This rather unexpected result seems at first difficult to explain, because mycelium in the marginal disks cut out and incubated sterile for one week at 25° C. would be almost identical in age with that in submarginal disks cut out one week later (for the reason that in one week at 25° C. the advance of the colony equals the diameter of the disk, 8.5 mm.: the incubation period of seven days was chosen for this reason).

The clue to this problem was afforded by observation of visible changes in the disks of series B and D after the week's incubation. Those of series D, left in their original positions after cutting, showed a much greater development of mycelium, as judged both by density of colour and by observation under the microscope, than those of series B, incubated separately in moist sterile dishes. Moreover, the disks of series D had to be recut at the end of the week's incubation, as they had been joined up again with the parent colony by renewed growth of mycelium. It is suggested, therefore, (i) that the greater effectiveness as inoculum of the disks of series D, and of submarginal disks in general, is due to a greater density of mycelium in such disks, (ii) that the greater density of mycelium in such disks is due not only to development of mycelium already within the disk area, but also to continued invasion of the disk area by hyphae from outside. This second conclusion may be stated in more general terms, as follows: *during one week's growth of a colony of Helicobasidium purpureum at 25° C., the original marginal zone of the colony is progressively invaded by hyphae from the submarginal zone.* The conclusion that development of mycelium within the marginal zone of a colony is due to infiltration of new hyphae from the submarginal zone, as well as to branching of the original hyphae within the marginal zone, is probably applicable to growth of fungus colonies in general.

One further comment is necessary on these results; the greater extent of

strand growth, both from marginal and submarginal disks, in this experiment than in Exp. I (Table 2) is to be ascribed to the shorter storage period of the tubers.

Experiment III. In spite of the employment of submarginal inoculum disks of 2 meat-2 malt agar, production and growth of mycelial strands in some subsequent experiments was still very variable. Several experiments on the effect of soil conditions on growth of mycelial strands were rendered worthless by the fact that many of the inoculum disks failed to produce any strands at all. In an endeavour to overcome this difficulty, a comparison was made between submarginal inoculum disks from plates poured at the normal depth of 3 mm. agar (20 ml. per plate) and at twice that depth (40 ml. per plate); a further comparison, between 2 meat-2 malt and 3 meat-3 malt agars was incorporated in this experiment. The plates were incubated after inoculation for four weeks at 25° C., and for a further week in the laboratory (mean temperature 19.5° C.). Ten tubers, variety King Edward, were inoculated on 5 April 1945 with each of the four types of submarginal inoculum disks, and buried in the same type of soil as in the previous experiments. Maximum growth of mycelial strands after nine weeks (mean laboratory temperature 17° C.) is recorded in Table 4.

Table 4. *Maximum growth of mycelial strands in mm.*

	Agar disks 3 mm. deep	Agar disks 6 mm. deep
2 meat-2 malt agar	5 ± 1.5	22 ± 4.3
3 meat-3 malt agar	15 ± 3.5	27 ± 4.0

The increase in maximum strand growth resulting from a double depth of agar is highly significant on the 2 meat-2 malt agar, but just fails to attain significance (by the *t* test) on the 3 meat-3 malt agar. Increase in strand growth with increase in nutrient concentration of the agar is significant only with the disks of shallow agar.

Experiment IV. It so happened that tubers for this experiment were inoculated one week later with submarginal disks taken from the same batch of plates as that used for Experiment III; the 2 meat-2 malt agar plates poured at double depth were selected for provision of inoculum. In all, ninety-six tubers, variety King Edward, were inoculated and kept for eight weeks (mean laboratory temperature 16° C.) in acid-treated (pH 5.5), untreated (pH 7.6) and limed (pH 8.0) Harpenden allotment soils at moisture contents of 40 and 60 % saturation (sixteen tubers for each series). Results of this experiment were extremely irregular and unsatisfactory; only 23/96 inoculum disks gave any strand growth at all, and only 18/96 gave a strand growth exceeding 10 mm. In the soils at 40 % saturation only 8/48 disks gave any growth, as against 15/48 disks for the soils at 60 % saturation, but the growth failures certainly could not be attributed to unfavourable soil conditions as the principal cause.

The most obvious difference between this experiment and the preceding one, in which better strand growth had been obtained, was that the plates for inoculation had been kept for one week longer in the laboratory at a

mean temperature of 19° C. The decreasing potency of the disks for strand production might therefore have been due to (i) increase of one week in age, (ii) transfer from the 25° C. incubator to a cupboard at laboratory temperature. A survey of all available experimental data showed that the unsatisfactory results from a number of experiments could be completely explained by the fact that plates for inoculum had been incubated for four weeks at 25° C., and then for a further one or two weeks in a cupboard. No evidence was available to show, however, whether the unsatisfactory behaviour of inoculum disks from these plates was to be attributed to increased age of the colony, or to some deleterious effect of the transfer from the incubator at 25° C. to a cupboard at laboratory temperature. It was decided to test first the effect of colony ageing at a continuous temperature of 25° C.

Experiment V. In this experiment, plates were poured at normal (3 mm.) and double (6 mm.) depth with three types of agar, 0 meat-2 malt, 2 meat-2 malt, and 3 meat-2 malt, and inoculated with *Helicobasidium purpureum* at such times that colonies of ages three, five and six weeks at a continuous temperature of 25° C. would be available for the cutting of submarginal inoculum disks on the same day. On 29 August 1945 ten tubers, variety Majestic, were inoculated with each type of inoculum disk; there were disks of three agars, of two depths and of three ages, in the eighteen possible combinations, making 180 inoculated tubers altogether. The same 1 soil : 3 sand mixture at 60% saturation was used as in previous experiments. Maximum strand growth after seven weeks (mean laboratory temperature 16° C.) is given in Table 5.

Table 5. *Maximum growth of mycelial strands in mm.*

Type and depth of agar	Age of colonies (weeks)		
	3	5	6
0 meat, shallow	41	43	26
0 meat, deep	40	47	48
2 meat, shallow	38	28	28
2 meat, deep	40	43	43
3 meat, shallow	32	33	25
3 meat, deep	40	45	44

Table 5 shows that although there is a decrease in maximum strand growth from the shallow inoculum disks taken from 6-week colonies, and even from those from 5-week colonies on the 2-meat agar, there is no such decrease in strand growth with increasing age of colony from the deep disks, and so these results are quite inadequate to account for those of Experiment IV. The observed decrease in strand growth from disks taken from progressively older colonies on shallow agar is probably due to production of staling substances by the ageing colony. Thus Brown (1923) showed that colonies of *Sphaeropsis malorum* and of species of a *Fusarium* staled more rapidly on shallow than on deep layers of agar, presumably because of the greater dilution of staling substances under the latter conditions.

The general high level of figures for strand growth in Table 5 is to be

attributed to the fact that tubers were still in the early stage of their storage life.

Experiment VI. As ageing of colonies at a continuous temperature of 25° C. was shown in the last experiment to be inadequate to explain the unsatisfactory behaviour of inoculum disks in Experiments III and IV, it seemed that this must be attributed to the taking of inoculum plates out of the incubator at 25° C., and holding them for a further one and two weeks, respectively, at laboratory temperature. To test this conclusion, a number of plates of 2 meat-2 malt agar, poured at normal depth (3 mm.), were inoculated with *Helicobasidium purpureum* and kept in the 25° C. incubator for three weeks. Half the plates were then left in the incubator for a further two weeks, and half were kept for the same period in a dark cupboard, the temperature of which varied from 13° to 19° with a mean of 16.5° C. during the first week, and from 14° to 19° with a mean of 16.5° C. during the second week. Forty tubers, variety Majestic, were then inoculated with disks from these plates on 18 December 1945, and buried in the 1 soil : 3 sand mixture at 60 % saturation. Maximum strand growth after seven weeks (mean laboratory temperature 15° C.) was 22 ± 1.9 mm. in the series inoculated with disks from plates held continuously at 25° C., and 7 ± 2.4 mm. in the other series. Evidently, therefore, the unsatisfactory behaviour of the inoculum disks in previous experiments was due not merely to ageing of the colonies, but to their transfer from the incubator at 25° C. to a cupboard at laboratory temperature for one or two weeks prior to cutting out of disks. Further research is required for elucidation of the mechanism of this effect.

In the meantime, it can be concluded from the results of these six experiments that, to secure best growth of mycelial strands, tubers should be in the early part of their storage life, and submarginal inoculum disks should be cut from plates held continuously at 25° C., preferably not more than three and a half weeks old. If these conditions are fulfilled, a 2 malt agar with any concentration of meat is adequate, but pouring of plates at double depth (6 mm.) is recommended as an additional safeguard, especially with older tubers.

Experiment VII. Having disposed of the difficulties described, it seemed possible to set up an experiment to determine the effect of soil conditions upon rate of growth of mycelial strands, with greater prospect of success. The experiment, of factorial design, comprised the eighteen possible combinations of three soil reactions, two textures, and three moisture contents. To secure soil of three different reactions, portions of moist Harpenden allotment soil were treated with 1.5 % sulphuric acid, and with 0.25 % calcium hydroxide, respectively; the treated soils, together with a third untreated portion, were incubated moist in 12 in. porous pots for eight weeks, and then air-dried and passed through the 14-mesh sieve. Two different soil textures were obtained by diluting part of each soil with three times its own volume of sand. Submarginal inoculum disks were cut from colonies on 2 meat-2 malt agar poured at double depth (6 mm.) and incubated at 25° C. for twenty-three days, and ten Majestic tubers were inoculated for each of the eighteen soil series on 10 September 1945;

optimum inoculum was therefore combined with the provision of tubers still in the early part of their storage life. Maximum strand growth (Table 6) was measured after a period of six weeks only, so that growth should be limited only by soil conditions, and not by reserves of the inoculum. Mean laboratory temperature during this period was 17° C.

Table 6. *Maximum growth of mycelial strands in mm.*

	Acid-treated soil	Untreated soil	Limed soil
Whole soil, 40 % saturation	12	20	27
Whole soil, 60 % saturation	17	23	27
Whole soil, 80 % saturation	23	18	19
Soil/sand, 40 % saturation	36	30	30
Soil/sand, 60 % saturation	34	32	32
Soil/sand, 80 % saturation	29	34	27
<i>pH values of soils</i>			
Whole soil, start of exp.	5.1	7.4	7.7
Whole soil, end of exp.	5.1	7.3	7.6
Soil/sand, start of exp.	5.1	7.5	7.8
Soil/sand, end of exp.	5.0	7.7	7.9

The most outstanding feature of these results is the increase in strand growth resulting from dilution of the soil with sand; this increase is highly significant. The other noteworthy feature is the retarded growth of strands in the acid-treated whole soil at 40 % saturation; this retardation may be ascribed to the joint action of three adverse factors, viz. acidity, low soil moisture, and a less than optimum soil texture. The aggregate effect of these three adverse factors acting together seems to be greater than the sum of their effects acting singly, as seen in other combinations of soil reaction, moisture content and texture. Thus under the favourable conditions of the soil/sand mixture acidity did not depress growth of strands at all (owing to the greater 'availability' of water in soil/sand mixture, moisture content would not be expected to limit strand growth at 40 % saturation). Again, in the whole soils, a decrease in soil moisture from 60 to 40 % saturation appreciably depressed growth only in the acid-treated soil, and not in the untreated or limed soils. These results recall those of Brown (1922), who found that toxic concentrations of carbon dioxide depressed the growth of fungi in culture most greatly when other conditions were also adverse, e.g. under conditions of low temperature and low concentrations of nutrients. Brown's general conclusion that 'the retarding factor has greatest effect when the energy of growth is small' helps to clarify, if not to explain, the results of the above experiment with *Helicobasidium purpureum*.

EXPERIMENTS WITH ROSSI-CHOLODNY SLIDES

The Rossi-Cholodny glass slides used for this and the following experiments measured approximately 8.2 × 5.4 cm., and were made by cutting old lantern slide glasses in half. In setting up the slides, part of the dry soil was first added to each jar and the slides fixed in their vertical position

before the soil was moistened. Inoculum disks were then placed, mycelial surface against the glass slides, in position just touching the surface of the moistened soil, and the remainder of the dry soil was then added, shaken down, and brought to the correct moisture content.

Experiment VIII. This first experiment was designed to compare growth of *Helicobasidium purpureum* along the slides with that over potato tubers, and was carried out in allotment soil, at a moisture content of 60% saturation. The inoculum disks were taken from the same plates, one day older, as those for Experiment VII. Ten slides and twenty tubers, variety Majestic, were inoculated; measurements of mycelial growth over the slides were made after three weeks, and of strand growth over the tubers after three and again after five and a half weeks (mean laboratory temperature 17° C.). Results are given in Table 7.

Table 7. *Maximum growth of mycelium in mm.*

	Slides	Tubers
After 3 weeks	27	11
After 5½ weeks	—	30

In the first three weeks, *Helicobasidium purpureum* grew more than twice as far over the slides as over the tubers. Although it is easier to detect mycelium on the slides than the apical portions of strands on the tubers, yet appreciable underestimate of strand growth is considered unlikely, because in the second period of two and a half weeks, strand growth over the tubers was twice as fast (by extrapolation, 23 mm. in three weeks as against 27 mm in three weeks over the slides). The apparently slower growth of strands over the tubers than of mycelium over the slides is therefore due chiefly to an initial check after inoculation of the tubers.

Experiment IX. Growth over the slides was compared in three lots of allotment soil, previously treated with 1% sulphuric acid, untreated, and previously treated with 0.25% calcium hydroxide, at three different moisture contents, 40, 60 and 80% saturation. Submarginal inoculum disks were taken from colonies aged three and a half weeks at 25° C., on plates poured at normal depth with 2 meat-2 malt agar. Maximum growth of mycelium over the slides after twenty-four days (mean laboratory temperature 16° C.) is given in Table 8.

Table 8. *Maximum growth of mycelium in mm.*

	Acid-treated soil	Untreated soil	Limed soil
40% saturation	16	26	24
60% saturation	17	26	27
80% saturation	21	31	25
<i>pH values of soil</i>			
Start of exp.	5.9	7.3	8.0
End of exp.	6.6	7.4	7.8

The depressing effect of soil acidity on growth of mycelium over the slides, to be observed in Table 8, was confirmed in a further experiment, in

which twenty slides were buried in acid-treated soil (1.25 % sulphuric acid: pH 5.6) and twenty in untreated soil (pH 7.3) at a moisture content of 60 % saturation. Growth of mycelium over the slides after twenty-four days (mean laboratory temperature 16° C.) was 19 ± 1.7 mm. in the acid-treated and 29 ± 0.9 mm. in the untreated soil.

SUMMARY

For this investigation, potato tubers were inoculated with 8.5 mm. disks cut from a growing colony of *Helicobasidium purpureum* on meat-malt extract agar, and buried in glass jars filled with soil; maximum growth of mycelial strands from the inoculum disk was measured after some seven weeks by a paper scale fitted to the curved surface of the tuber.

The distance covered by mycelial strands and the frequency of infection cushions decreased with increase in storage age of the tuber. Inoculum disks cut from the growing margin of the fungus colony (marginal disks) were less effective for production and growth of strands than disks cut within a circle drawn 8.5 mm. from the margin (submarginal disks). The greater effectiveness of submarginal disks was related to their greater mycelial density; progressive development of mycelium within the marginal zone of the fungus colony was due to infiltration of new hyphae from the submarginal zone, as well as to branching of the original hyphae within the marginal zone. Production and growth of strands also increased with concentration of nutrients in the agar, and especially with that of the malt constituent. With increase in age of the colony to five or six weeks at 25° C., the potency of inoculum disks for strand production declined, but this apparent staling effect did not occur with disks cut from colonies on double depth agar (6 mm.). Transfer of colonies after three or four weeks' growth at 25° C. to laboratory temperature (15–20° C.) for a further 1–2 weeks reduced strand growth from disks more severely than did continued incubation of the colonies at 25° C. for the same period.

Growth of strands was greater in a 1 soil: 3 sand mixture than in soil. Soil acidity depressed strand growth at medium and low but not at high soil moisture content, and not in the soil/sand mixture. This adverse effect of soil acidity was also shown in growth of mycelium over Rossi-Cholodny slides.

I am indebted to Mr W. Buddin and Dr R. Hull for valued assistance, and especially for enabling me to see occurrences of violet root rot.

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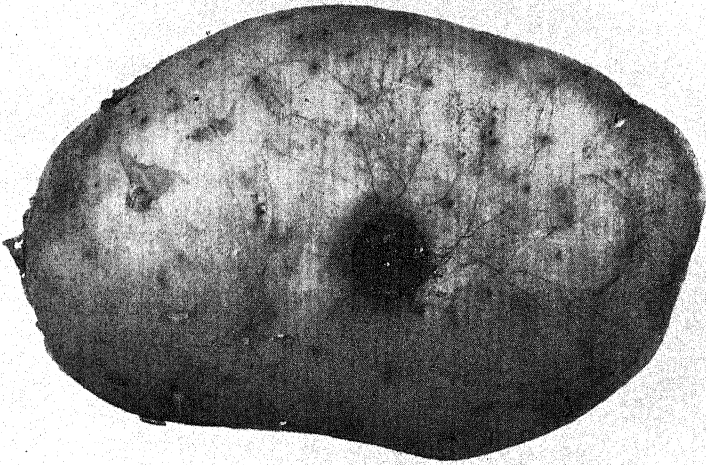


Fig. 1

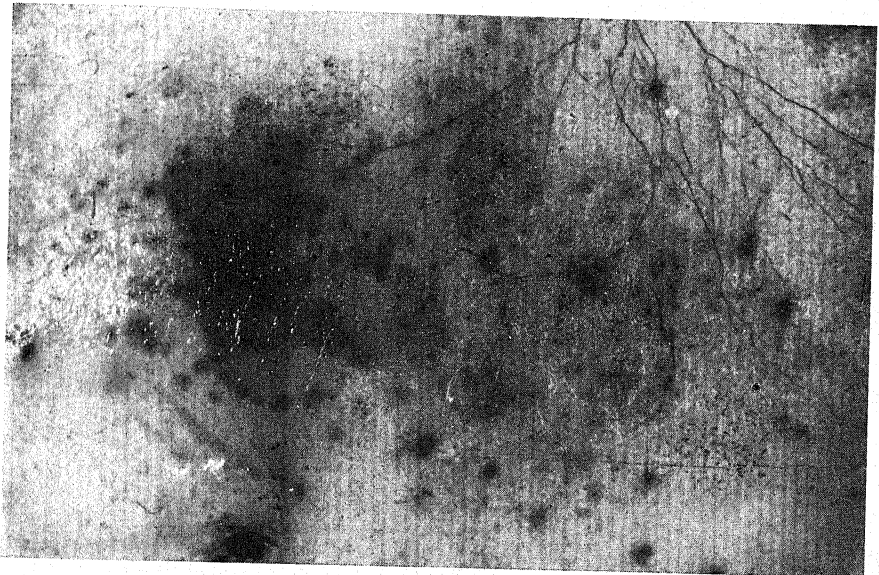


Fig. 2



Fig. 1



Fig. 2

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EXPLANATION OF PLATES

PLATE V

- Fig. 1. Potato tuber inoculated with agar disk of *Helicobasidium purpureum*, showing growth of mycelial strands.
- Fig. 2. Surface of tuber inoculated with *H. purpureum*, showing mycelial strands and infection cushions (visible as dark points distributed over areas of local discoloration).

PLATE VI

- Fig. 1. Progressive infection and rotting by *H. purpureum*, in two potato tubers collected in field by Mr W. Buddin.
- Fig. 2. Epiphytic strands of *H. purpureum* on surface of potato tuber collected in field by Mr W. Buddin.

(Accepted for publication 5 April 1946)

STUDIES ON BRITISH CHYTRIDS

I. *DANGEARDIA MAMMILLATA* SCHRODER

By HILDA M. CANTER, B.Sc.

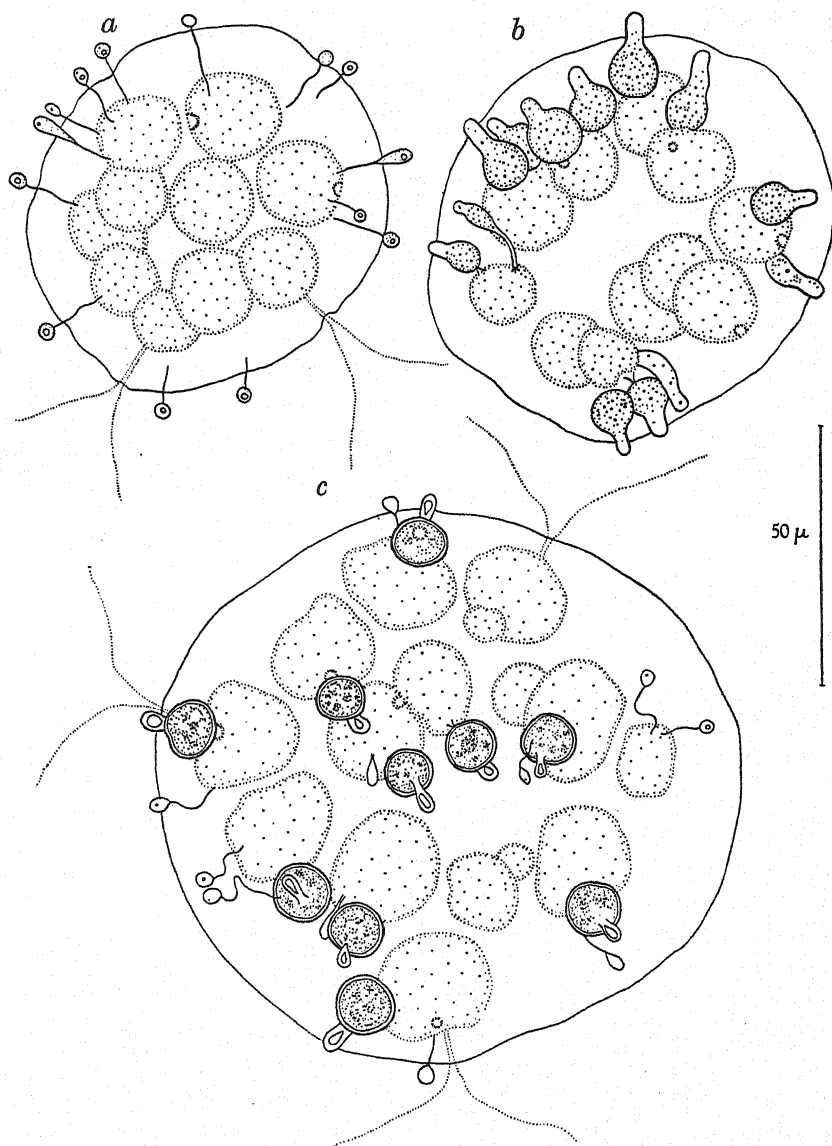
Department of Botany, Queen Mary College, London

(With Plate VII and 5 Text-figures)

In the plankton of Barn Elms Reservoir (No. 5), Hammersmith, London, *Eudorina elegans* Ehrenb. was found from November 1945 to January 1946 to be infected by a chytridiaceous fungus bearing a striking resemblance to *Dangeardia mammillata* which Schröder (1898) originally described on *Pandorina morum* Bory. A detailed study was made of the life history of this fungus based entirely on observations from living material.

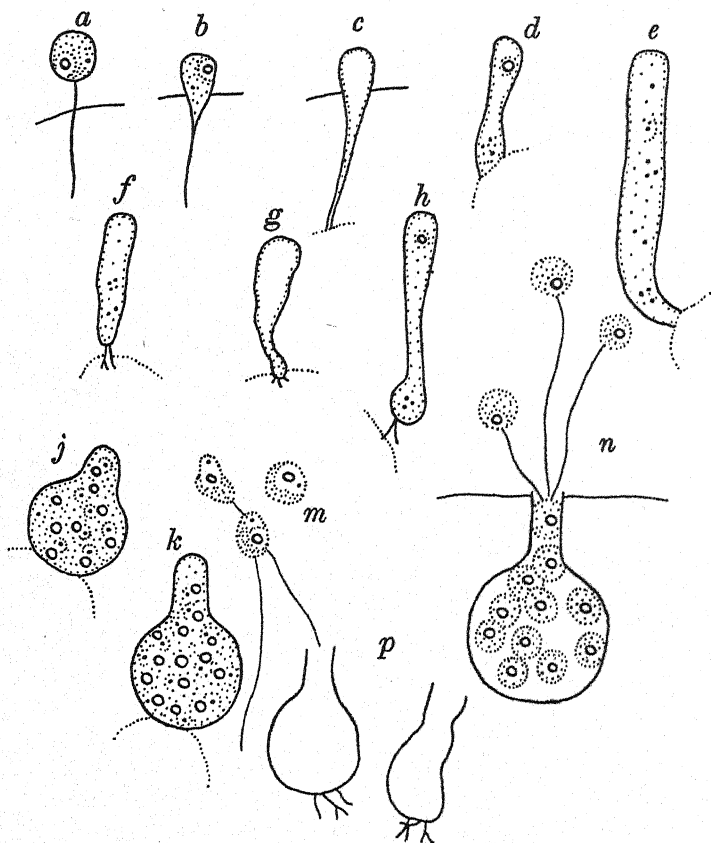
In the parasitized coenobium the chloroplast of each infected cell contracts away from the wall, and is finally reduced to a small mass of yellowish brown granules. As many as thirty individuals of the parasite are sometimes to be seen in a single coenobium, but, provided that one or two cells remain unaffected, the host retains its motility.

The story of development of the parasite has been built up by the examination of a large number of individuals in varying stages. The zoospore encysts on the surface of the coenobium and produces a fine germ tube which grows through the mucilage sheath to the nearest host cell (Text-fig. 1*a*). After contact is made with a host cell the germ tube gradually broadens from the proximal towards the distal end, until the thallus is almost cylindrical. The base then continues to swell, and the developing sporangium becomes flask-shaped, embedded, except for the extreme apex of the neck, in the gelatinous sheath of the *Eudorina* coenobium (Pl. VII, fig. 1). The mature sporangia range in size from 7 to 15 μ in diameter by 10 to 24 μ in length. In several examples, before the germ tube had become equally broad along all its length, the basal swelling had already begun to develop and bore a few rhizoids. The rhizoids are numerous, short, usually unbranched structures (3 μ in length) arising in a tuft from the base of the sporangium and penetrating the host cell (Text-fig. 2*f*, 3, 4, 5). The changes in the protoplasm during spore development are similar to those described for most chytrids, and at maturity the sporangium contains a number of similar, and equally spaced, refractive globules, each indicating the position of a zoospore. A mature sporangium may produce from twenty to one hundred zoospores which emerge singly, squeezing through the opening formed upon deliquescence of the apex (Text-fig. 2*n*). Immediately the first zoospores have emerged the remainder begin to move over one another, and a swarming mass of them is to be seen in the sporangium which empties, however, within 5-10 min. after dehiscence. The empty sporangium does not collapse immediately, but fairly soon shows signs of shrivelling.



Text-fig. 1. *Dangeardia mammillata*. *a*, young thalli each consisting of an encysted zoospore and thread-like germ tube. *b*, a number of nearly mature sporangia. *c*, resting spores, some with associated male thalli. All $\times 700$.

The zoospores appear to be of two types, and each sporangium produces one kind only. The sporangia themselves are morphologically indistinguishable. Some zoospores are spherical, 2.5μ in diameter with a single conspicuous oil globule surrounded by an area of less dense protoplasm, and have a single posterior flagellum 15μ long. Others have, in addition to the oil globule, a minute rod-shaped oscillating granule in an anterior

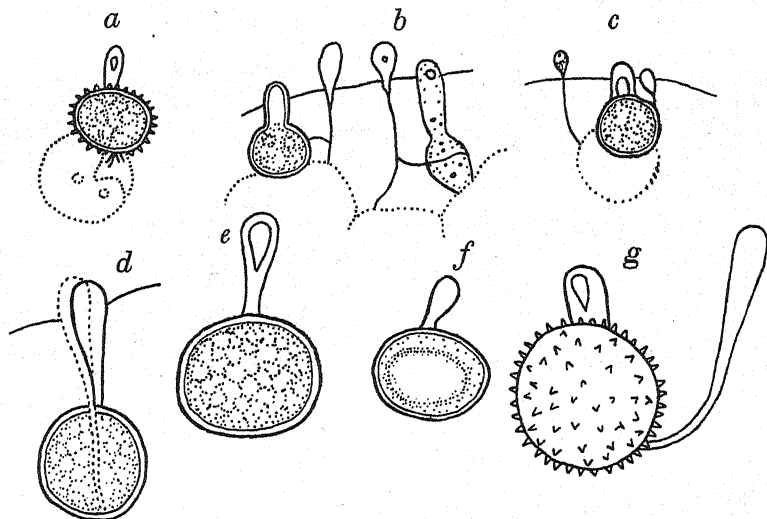


Text-fig. 2. *Dangeardia mammillata*. a-h, stages in early development of thallus. j, k, mature sporangia, each oil globule indicates the position of a zoospore. m, zoospores with oscillating granule in addition to the oil globule. n, zoospores with oil globule but no granule, escaping from sporangium. p, two empty sporangia showing rhizoids. d, $\times 1333$; the rest $\times 1777$.

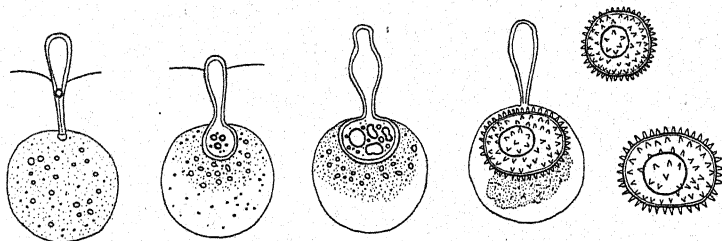
position (Text-fig. 2m). These zoospores are often somewhat oval ($3.5 \times 1.8\mu$), and appear to be slightly flattened.

In collections made during November zoosporangia only were observed, but by mid-December sexually formed resting spores had appeared (Text-fig. 3, and Pl. VII, figs. 2-4). In resting-spore formation, union occurs between a relatively large flask-shaped thallus (presumably the female), almost identical with a zoosporangium, and a relatively small one (pre-

sumably the male), resembling an early stage in the development of an asexual sporangium. There were certain strong indications that the male thalli were derived only from zoospores of the type with a moving granule, but further work in single-spore cultures seems necessary before such a remarkable state of affairs can be established.



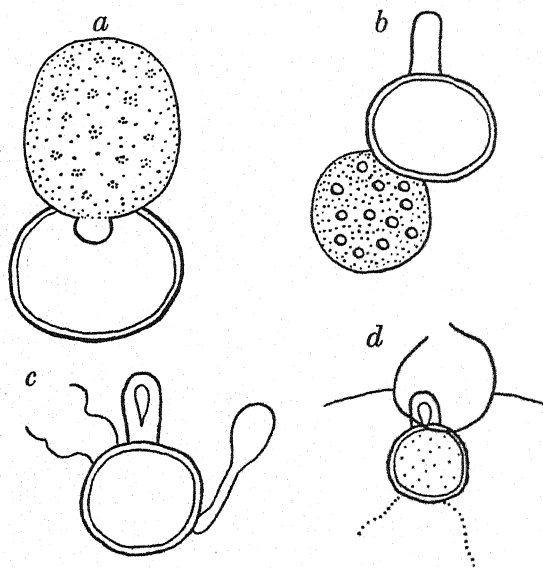
Text-fig. 3. *Dangeardia mammillata*. *a*, spiny resting spore with rhizoids, $\times 933$. *b*, two young resting spores with associated male thalli, having branched germ tubes, $\times 1333$. *c*, smooth-walled resting spore with two males; the one on the right still retains some of its contents, $\times 933$. *d*, *e*, resting spores showing clavate appendage, derived from the zoospore and its germ tube, $\times 1777$; in *d*, the male thallus is drawn with a broken outline. *f*, resting spore with a single large oil globule, $\times 1300$. *g*, spiny-walled spore with male thallus, $\times 1777$. In figs. *d*, *f*, the appendage is completely filled with refractive material; in figs. *a*, *c*, *e* and *g*, a centrally unthickened area remains.



Text-fig. 4. Stages in the development of a resting spore of *Dangeardia mammillata*, according to Schröder (1898).

The young resting spore can be recognized not only by the presence of an associated male thallus (Pl. VII, fig. 4), but also by the clavate germ tube and by the appearance of the oily contents. The germ tube of the male thallus makes direct contact with the swollen base of the female, and the empty

male normally connected with each mature resting spore indicates that the contents of the male pass into the female. Further evidence was afforded by the fact that where two males were associated with a female thallus one was empty, whereas the other still retained its contents. The germ tube of the male thallus was very rarely seen to branch (Text-fig. 3*b*). While a male thallus was found connected with most of the resting spores, it appeared to be absent from a few, and these spores may have developed parthenogenetically. As development proceeds, presumably following fusion, the wall of the neck of the female thallus begins to thicken from the outside towards the centre. A solid plug of highly refractive material is normally produced, but in some specimens, which were perhaps immature,



Text-fig. 5. *Dangeardia mammillata*. *a*, germinated resting spore with immature germ-sporangium on its surface, $\times 1777$. *b*, germinated resting spore with almost mature germ-sporangium, each oil globule indicates the position of a zoospore, $\times 1777$. *c*, germinated resting spore with associated male thallus and empty shrivelled wall of the germ-sporangium, $\times 1777$. *d*, empty resting spore with granulated wall, the granules on the surface of the wall are visible after germination, $\times 1300$.

a centrally unthickened area remained (Text-fig. 3*e, g*). The length of this neck varies, and it is often characteristically narrowed towards its attachment to the swollen base of the female thallus (Pl. VII, fig. 3), which forms the actual resting spore. The latter at maturity is oval to spherical in shape, $7.5\text{--}13\mu$ in diameter, and has a thick wall which may be smooth, granular or distinctly spiny. The contents are usually oily with numerous scattered droplets, but several specimens were seen with a single large oil globule (Text-fig. 3*f*). The zygote germinates readily under laboratory conditions. A thin-walled sporangium is formed on the surface of the resting spore, which is left without protoplasmic contents, thus behaving as a pro-sporangium in germination (Text-fig. 5*a, b*). Actual dehiscence of these

sporangia was not seen, but from observations on mature and dehiscid specimens from which the zoospores were still escaping, it is clear that posteriorly uniflagellate zoospores are produced. It also seems that as in the zoosporangia, the germ-sporangia produce zoospores all of one type, either with a single oil globule or with an additional oscillating granule. Zoospores with an oscillating granule are not unknown among chytrids. Hanson (1944) describes such zoospores as typical of *Loborhiza Metzneri*, a parasite on *Volvox*.

The type of sexuality exhibited by *Dangeardia* most nearly resembles that described for *Zygorhizidium Willei* Löwenthal. In the latter the male thallus similarly produces a conjugation tube which makes direct contact with a swollen female thallus.

Since *Dangeardia mammillata* was originally described by Schröder (1898) few references to it have appeared in the literature. Skvortzow (1927) gives a brief description of a fungus from Manchuria which he identified with *Dangeardia*, but since he observed neither the rhizoids nor the resting spores, and the zoospores were more than twice the diameter of those described by Schröder, the identity of this chytrid remains uncertain. Ingold (1940) found what he believes to be the same organism parasitizing *Eudorina elegans* in the plankton of Swithland Reservoir, Leicestershire, England. He did not, however, observe the resting spores (private communication). Sparrow (1943) recorded it from America as a citation from a personal communication with Bartsch. Bartsch (*in lit.*) indicated that his observations agreed with those of Schröder.

Although the chytrid described in this paper agrees well with *Dangeardia mammillata*, it nevertheless departs from the original description in one major aspect, namely, that the resting spores are epibiotic and sexually formed. That Schröder (see Text-fig. 4) may have interpreted an epibiotic resting spore as endobiotic is not unlikely, for a spore, when viewed immediately above a host cell (especially if the chloroplast is rather shrunken), often gives the appearance, at first sight, of being endobiotic. Other characteristics of the resting spore as described by Schröder were the clavate appendage (derived from the zoospore and its germ tube), the spiny wall, and single oil globule. All these features have been observed in the material examined by me (see Text-fig. 3, and Pl. VII, figs. 2, 3), leaving little doubt as to the identity of the fungus.

Hood (1910) described *Rhizophyidium Eudorinae*, a parasite on *Eudorina elegans*, which in its method of development, shape, size and inoperculate nature of the sporangia, and size of the zoospores agrees with *Dangeardia*. However, she saw no rhizoids and the zoospores were described as emerging in a mass embedded in mucilage. The spherical resting spore which Hood associated with *Rhizophyidium Eudorinae* is unlike that of *Dangeardia*, but may possibly belong to another fungus. Her figures of *Rhizophyidium Eudorinae* suggest that at least two chytrids were present on the *Eudorina*. An epibiotic species is shown in her paper (1910, Text-fig. 5) under abnormalities of *Rhizophyidium Eudorinae*.

Dangeardia mammillata seems to differ sufficiently from other chytrids to justify its retention in a separate genus, but an alteration in the definition

of the genus is necessary, since the resting spore is epibiotic, and not endobiotic as originally described. It is further suggested that *Rhizophyidium Eudorinae* is not a valid species, but would seem to represent material of *Dangeardia mammillata* together with an epibiotic chytrid belonging possibly to *Rhizophyidium* or *Chytridium*.

My thanks are due to the Metropolitan Water Board for permission to collect samples of plankton from Barn Elms Reservoir, and especially to Prof. C. T. Ingold for the helpful advice and criticism he has given throughout the course of this work.

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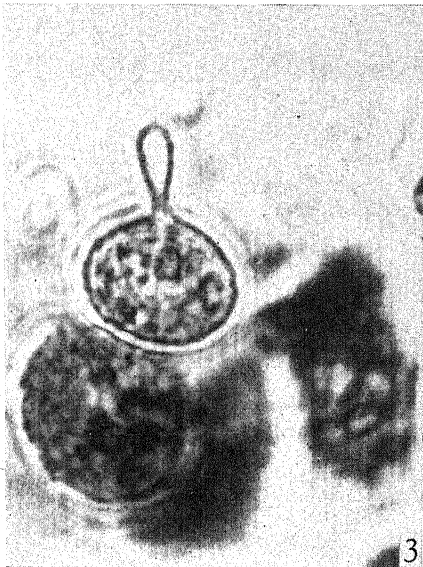
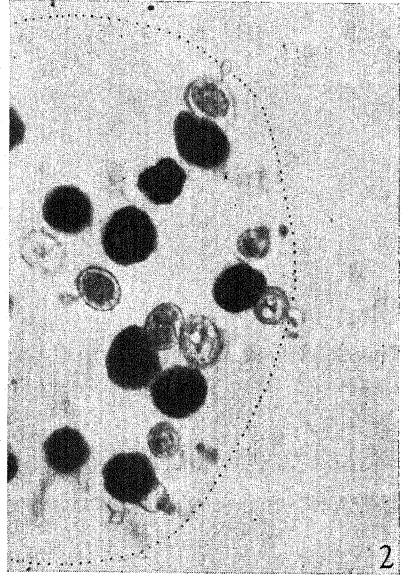
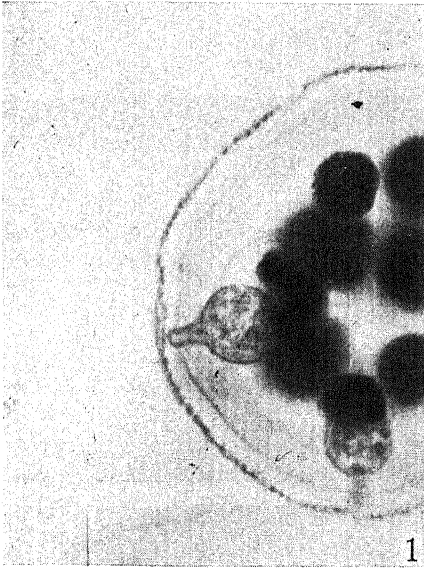
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EXPLANATION OF PLATE VII

Dangeardia mammillata Schröder

- Fig. 1. Part of a *Eudorina* colony showing two sporangia. $\times 1300$.
Fig. 2. Part of a *Eudorina* colony with resting spores; one near the centre shows a single large oil globule. The mucilage sheath of the host is indicated by a dotted line. $\times 900$.
Fig. 3. Resting spore with granular wall and clavate appendage filled with refractive material. $\times 2500$.
Fig. 4. Above resting spore, below empty male thallus; contact between them is obscured by the dense contents of the host chloroplast. $\times 2666$.

(Accepted for publication 17 April 1946)



NEW CHYTRIDIACEOUS FUNGI FROM CAMBRIDGE

By R. H. HASKINS

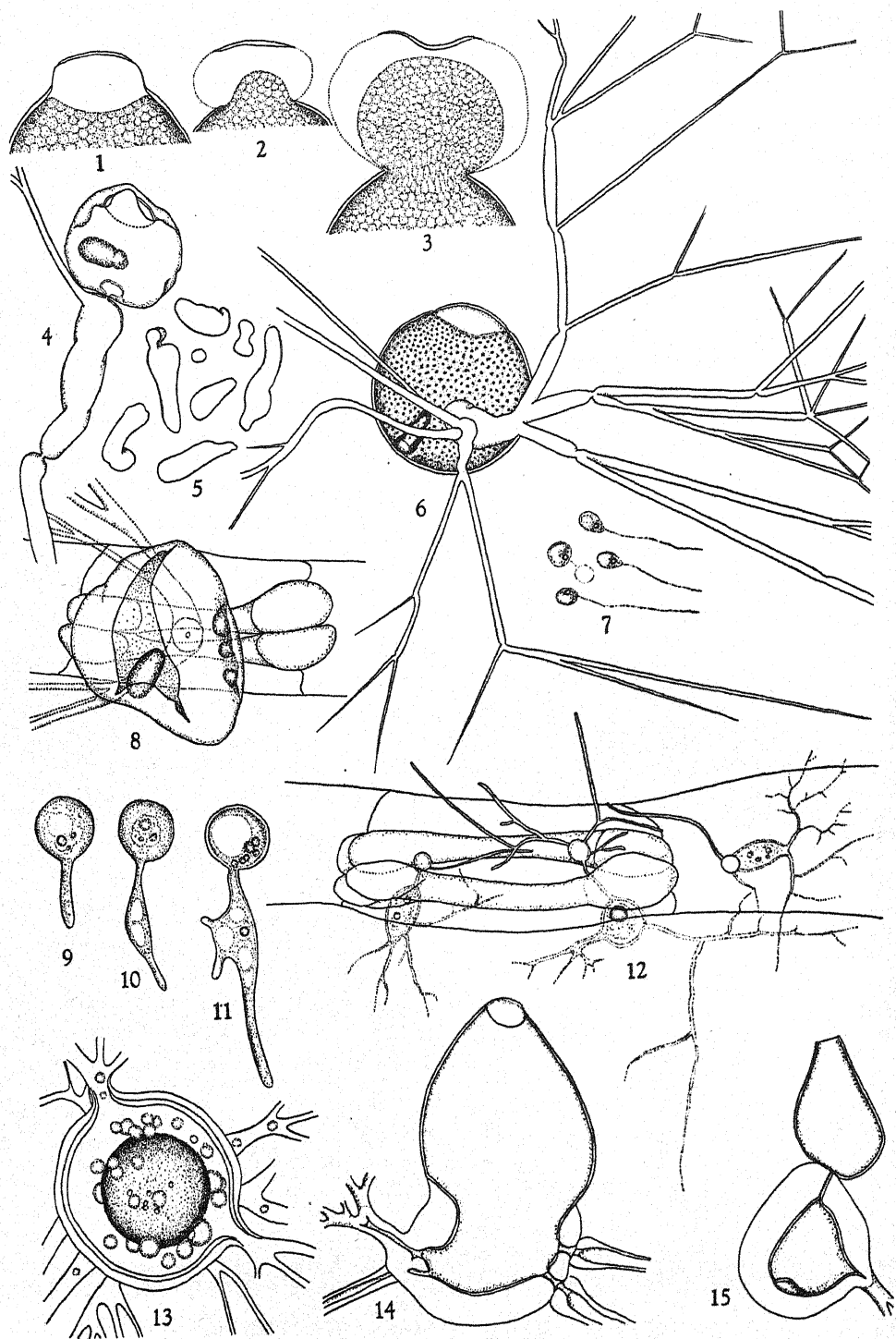
(With Plate VIII and 15 Text-figures)

In July 1945, collections of saprophytic chytrids were made in Cambridge, England, and its vicinity. Boiled hempseed, dechlorophyllized grass, maize and wheat leaves, filter paper and regenerated cellulose film, serving as baits, were placed in various ditches and ponds. For convenience in retrieving the baits, they were placed in muslin or gauze bags and held under water in small wire cages. After from one to three weeks, the baits were brought into the laboratory and examined. In some pieces, the presence of saprophytic chytrids could be detected at once. All baits were carefully washed in a gentle stream of tap water, and placed in sterile Petri dishes containing sterile charcoal water (distilled water filtered through animal charcoal, then autoclaved), and fresh pieces of bait added.

These gross cultures were examined from time to time and a number of species of chytrids and other aquatic fungi were identified. Many of the fungi identified have already been reported for the area by Sparrow (1936) and therefore will not be mentioned here. Species of the following genera were set aside for future study: *Olpidium* (in rotifers), *Olpidiopsis* (in *Achlya* and *Saprolegnia*), *Lagenidium* (in desmids), *Rhizidiomyces* (on insect exuviae and similar to *R. Hansonii* Karling (Karling, 1944)), *Diplophlyctis* (on grass and regenerated cellulose film), *Cladochytrium* (in grass, *C. hyalinum* Berdan (Berdan, 1941)), and *Nowakowskiella* (on grass and regenerated cellulose film, *N. hemisphaerospora* Shanor). Four of the forms collected, however, were given special attention, and two are briefly described in this preliminary paper.

Using methods and technique described by Berdan (1939) and Haskins (1939a), the new forms were brought into unifungal culture. Unifungal cultures are satisfactory for morphological descriptions, and the writer therefore made no attempt to procure 'pure' cultures. Such work is now in progress following the methods of Stanier (1942). The cultures from which these studies were made were unisporangial.

One of the forms (Text-figs. 1-8) was at first wrongly identified as *Rhizidium ramosum* Sparrow. Closer examination proved the sporangia to be operculate (Text-figs. 1-3). It cannot be included in the operculate genus *Chytridium* because its zoosporangia do not develop endo-exogenously (see Karling, 1936), but develop directly from the body of the encysted zoospore. It was therefore considered advisable to erect a new genus to include monocentric, operculate, rhizidiaceous chytrids in which the zoosporangia arise from the enlargement of the encysted zoospore and in which the resting sporangium is epibiotic or extramatrical. A paper was prepared describing and naming the genus and the type species. When the



Text-figs. 1-15

description of the new genus *Chytriomyces* (Karling, 1945) was received from America towards the end of August, it was immediately obvious that the genera were identical, and a revision of the present paper was undertaken. The fungus described in this paper is sufficiently different from the two species for which Karling erected the genus to be considered new. The species is characterized by variously shaped, refractive nodules (Text-figs. 4-6, 8) that are borne on the interior surface of the zoosporangial wall, and is accordingly named *Chytriomyces nodulatus*.

Chytriomyces nodulatus sp. nov. Zoosporangio hyalino, sphaerico, 15-50 μ dia. (plerumque 33 μ), cum tumoribus 1-20 nodosis, hyalinis, refractivis, variatim formatis, a pariete sporangiali alioqui levissimo ad centrum eminentibus, exteriora simul parietis nonnunquam irregularia efficientibus; interioribus granulatis, globulis aequae plerumque distributis, spatio sub ipso operculo vacuo. Operculo apiculato-subapiculato, brevi, neque in vacuo sporangio permanente; orificio circulari 12-18 μ dia., pariete sporangiali saepe post dehiscientiam scisso. Subsporangiali tumore variatim formato vel absente, ad 10 μ dia., a maturo sporangio pariete quodam intercepto. Rhizoideo systemate (addito subsporangiali tumore) 30-300 μ magnitudine. Rhizoidibus tenuissimis, 0.5-5.0 μ dia., ramis dichotomis, nonnunquam propter obstantia constrictis. Zoosporis partim adultis primum eiectionis, deinde in vesicula evanescenti compressis; hyalinis, ovatis-pyriformibus, 3.0-4.5 μ , uninucleatis, cum uno (rarissime compluribus) globulo refractivo 1.1 μ dia. prope nexum flagelli; uniflagellatis; flagello sex vel octo partibus zoosporo longitudine maiore, cauda praedito. Infuso ZnCl₂ interiora parietis zoosporangialis paululum temporis suppurea manent; spatium illud sub rubro operculo vacuum neutrali intra-vitam rubescit rubore, donec dehiscit. Sporangia perdurantibus suffuscis, levibus, sphaericis, 10-35 μ dia., cum uno vel compluribus magnis, minoribus parietalibus globulis, exstantibus prosperangiis ad germinationem.

Chytriomyces nodulatus n.sp. Zoosporangia hyaline, spherical, 15-50 μ in diameter (average 33 μ), with one to twenty hyaline, refractive, variously shaped, nodular protuberances extending centripetally from the otherwise smooth sporangial wall, and sometimes causing irregularities on the outer surface; contents granular with more or less evenly distributed globules; area immediately under the operculum clear. Operculum apical to subapical, shallow, not persistent on the empty sporangium; orifice circular,

Legends to Text-figs. 1-15

- Text-figs. 1-8. *Chytriomyces nodulatus*. Figs. 1-3. Stages of dehiscence of zoosporangium showing operculum and vesicle. $\times 924$, 693, 785 respectively. Fig. 4. Emptied zoosporangium with nodule. Note invaginated cross-wall separating zoosporangium from rhizoidal system. $\times 693$. Fig. 5. Nodules from various sporangia. $\times 739$. Fig. 6. Mature thallus. $\times 485$. Fig. 7. Zoospores. $\times 785$. Fig. 8. Emptied sporangium over stoma of substratum. Note splitting of zoosporangial wall, nodule, and dichotomy of subsporangial swelling. $\times 693$.
- Text-figs. 9-15. *Entophlyctis aurea*. Figs. 9-11. Three successive stages in germination of zoospore. $\times 1663$. Fig. 12. Young stages of thallus development showing infection through stomatal pore and directly through substratum wall. $\times 462$. Fig. 13. Resting sporangium. $\times 601$. Figs. 14, 15. Germinating resting sporangia. Drawings made with camera lucida. $\times 739$.

12–18 μ in diameter, sporangial wall often splitting after dehiscence. Subsporangial swelling variously shaped or absent, diameter up to 10 μ , cut off by a cross-wall from the mature sporangium. Rhizoidal system including subsporangial swelling 30–300 μ in extent. Rhizoids delicate, from 0.5–5.0 μ in diameter, branching dichotomous with constrictions where passing through obstructions. Zoospores, partially developed, discharged into, and later swarming in, an evanescent vesicle; hyaline, oval to pyriform, 3.0–4.5 μ , uninucleate with single (rarely several) refractive globule 1.1 μ in diameter near point of attachment of flagellum; uniflagellate, flagellum six to eight times diameter of zoospore in length, with tail-piece. Treatment with chloriodide of zinc producing short-lasting, extremely faint mauve coloration of inner surface of the zoosporangial wall; with intra-vitam neutral red operculum staining red, clear zone beneath operculum reddening, until dehiscence. Resting sporangia brownish yellow, smooth, spherical, 10–35 μ in diameter with one or several large globules and parietal smaller ones; functioning as pro-sporangia in germination.

Saprophytic in leaves of wheat, maize, oats and various grasses, and in insect exuviae, submerged in water, Cambridge, England.

The second of the forms (Text-figs. 9–15 and Pl. VIII) was the large, monocentric, orange-coloured, rhizidiaceous chytrid previously described as *Rhizophlyctis* (?) *Petersenii* Sparrow by Haskins (1939*a*, 1939*b*). Careful investigation of the early stages of development showed that the sporangium develops from a swelling on the germination tube and not directly from the body of the encysted zoospore. Attempts to grow this form on insect exuviae on which Sparrow (1937) found *R. Petersenii* were unsuccessful. This chytrid is now placed in its correct genus *Entophlyctis*, and because of the distinct bright orange colour of the mature sporangium is named *Entophlyctis aurea*.

Entophlyctis aurea sp. nov. Zoosporangiis plerumque intramatrixlibus, sphaericis, vel variatim formatis, 15–470 μ dia.; interioribus luteis-subluteis iuvenilibus, croceis maturis; pariete hyalino et stratificato, strati intimi reactione cellulosa (ZnClI) conspicua; strato extremo haud fucato. Tubulis exeuntibus 1–10, curtis, marginibus reflexis, 4.2–21 μ dia. ad orificium, 5.3–35.7 μ ad basin, 6.3–50.3 μ altitudine, gelatina clausis. Rhizoidibus locis 1–20 e sporangio eminentibus, robustis, ad 63 μ dia. ad originem, a maturo sporangio parietibus cellulosis interceptis, ramosissimis, ad 750 μ long. Zoosporis sphaericis-sub-sphaericis, 4.5–5.2 μ dia., cum compluribus granulis vel globulis parvis refractivisque; uniflagellatis; flagello quinque partibus zoosporo maiore longitudine; zoosporis permultis, emergentibus initio per unum tubulum exeuntem in massam sphaericam vel irregularem, primum quiescentibus, deinde enatantibus, reliquis zoosporis per cunctos effugientibus tubulos, labentibus vel emicantibus, interdum amoebaeis. Sporibus perdurantibus phora formaque zoosporangiis similibus, 22–106 μ dia., interioribus cum singulis vel compluribus croceo-fuscis globulis (11.4–60.8 μ dia.), fortasse cum minoribus in peripheria globulis sine colore; pariete crasso (3–10 μ), hyalino, stratificato, strati interioris reactione cellulosa in germinatione conspicua;

prosporangii vice fungentibus in germinatione ut surgat per porum exiguum zoosporangium extramaticale evanescens, vel per hiatum latis-simum zoosporangium sacculo simile.

Entophlyctis aurea n.sp. Zoosporangia usually intramatrix, spherical or variously shaped, 15–470 μ in diameter; contents yellow to pale orange in colour when young, bright orange at maturity; wall hyaline and layered, innermost layer giving marked cellulose reaction with chloriodide of zinc, outer layer not staining. Exit tubes one to ten, short with reflexed rims, 4.2–21 μ in diameter at orifice, 5.3–35.7 μ at base, 6.3–50.3 μ in height, with gelatinous plugs. Rhizoids arising from one to twenty places on the sporangium, stout, up to 63 μ in diameter at point of origin, cut off from mature sporangium by cellulose cross-walls; much branched, extending up to 750 μ in length. Zoospores spherical to subspherical, 4.5–5.2 μ in diameter, with several small refractive granules or globules; uniflagellate, flagellum five times diameter of zoospore in length; number of zoospores great, emerging initially through one exit tube to form a spherical or irregular mass, lying quiescent for a few moments before swimming away, remaining zoospores escaping through all exit tubes; movement gliding and darting, intermittently amoeboid. Resting spores borne and shaped similarly to zoosporangia, 22–106 μ in diameter, contents with one to several large orange-brown globules (11.4–60.8 μ in diameter), with or without smaller parietal colourless globules; wall thick (3–10 μ), hyaline, layered, inner layer at germination giving marked cellulose reaction; functioning as prosporangium in germination to give rise through a narrow pore to an extramatrix evanescent zoosporangium, or through a wide opening to a sac-like zoosporangium.

Saprophytic in various grasses, wheat, oat, and maize leaves, regenerated cellulose film, filter paper, and lens paper submerged in water, North Bay, Ontario, and London, Ontario, Canada (Haskins, 1939a), and Cambridge, England.

SUMMARY

A new species, *Chytrium nodulatus*, is proposed for a chytrid of the genus *Chytrium*. This species is characterized by variously shaped, refractive nodules borne on the inner surface of the sporangial wall. It occurs saprophytically in leaves of grasses and cereals, vegetable debris, and insect exuviae submerged in water.

A new species, *Entophlyctis aurea*, is suggested for a large, monocentric, inoperculate, rhizidiaceous chytrid with bright orange contents in the mature sporangium. It is extremely variable in size and shape.

Both of these chytrids have been separated from other aquatic fungi found in baiting material placed in ponds and ditches near Cambridge, England, and have been subsequently cultured in England in unifungal state under as nearly sterile conditions as possible.

The author expresses his sincere thanks to Prof. F. T. Brooks, Botany School, Cambridge University, for placing at his disposal the laboratory and library facilities of the School, and for his many kindnesses and

encouraging interest; and to Dr J. D. Ralph, London, Canada, for the Latin diagnoses. The work was done while awaiting repatriation, on leave of absence from the Canadian Army Overseas. Author's present address: Department of Botany, University of Western Ontario, London, Ontario, Canada.

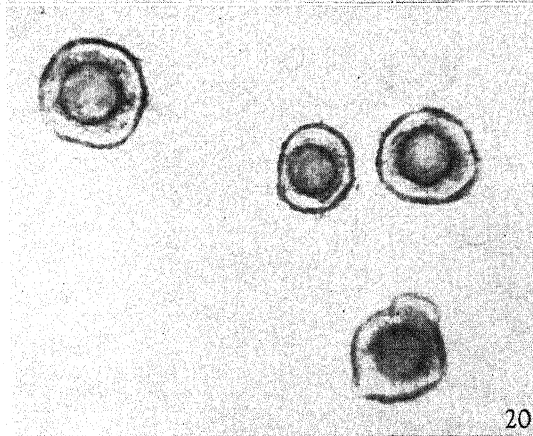
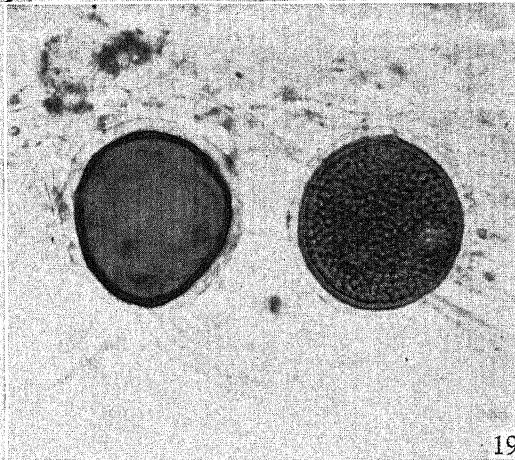
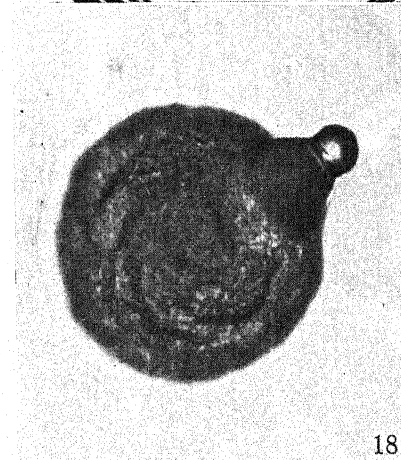
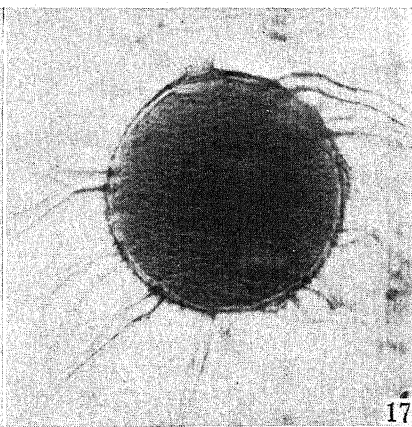
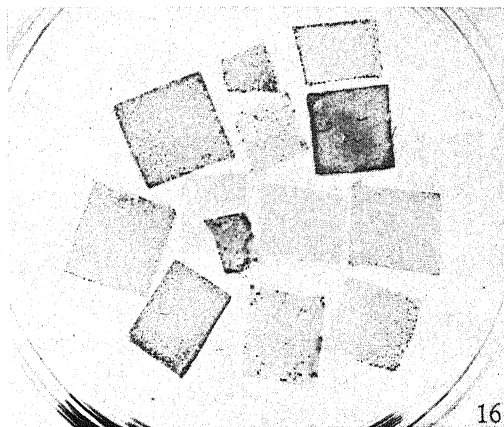
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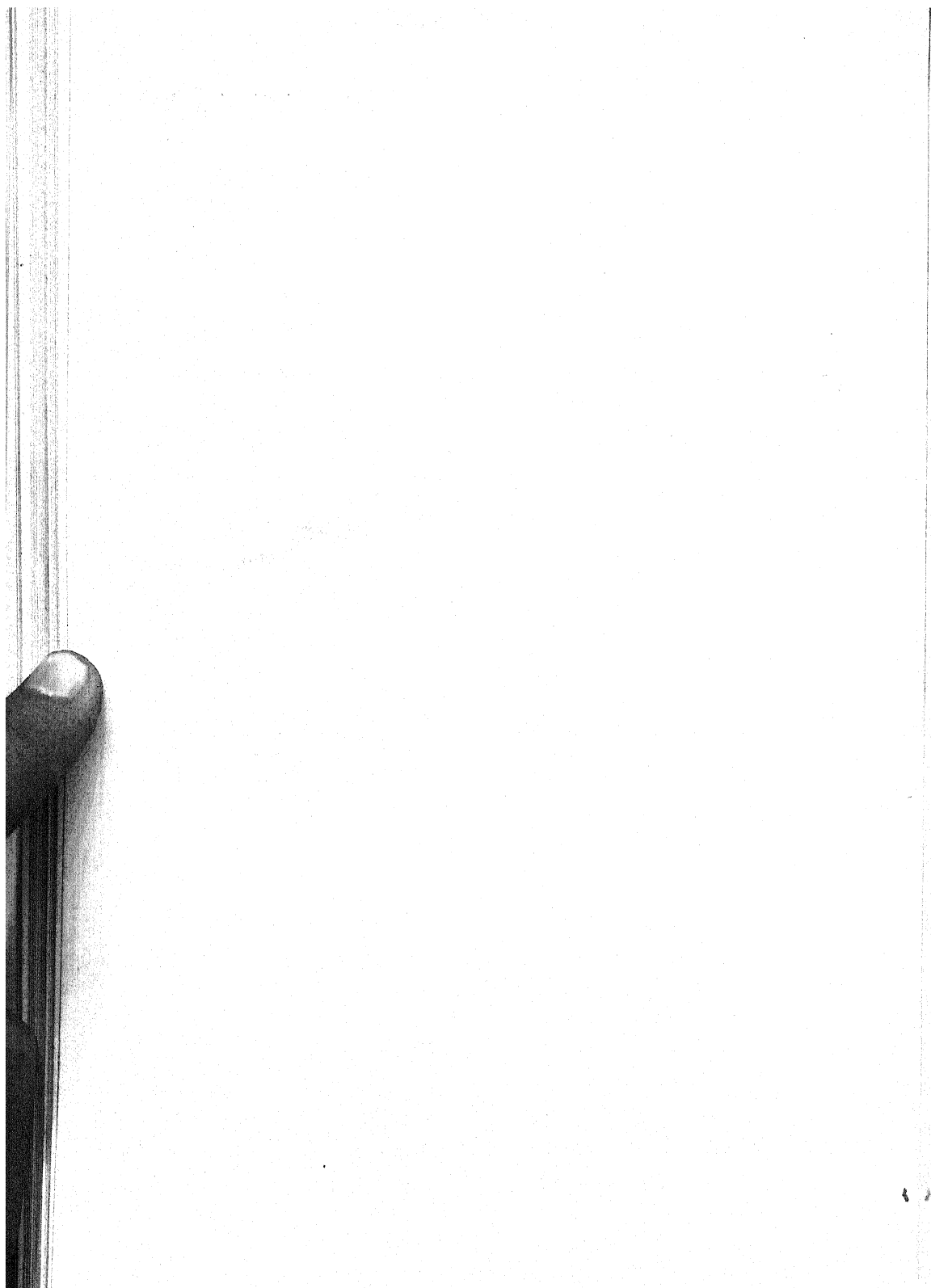
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EXPLANATION OF PLATE VIII

Figs. 16-21. *Entophlyctis aurea*. Fig. 16. Culture on regenerated cellulose film. Black dots are individual zoosporangia. From Kodachrome photomicrograph. $\times 0.76$. Fig. 17. Mature spherical zoosporangium from culture on regenerated cellulose film. Note stout rhizoids and gelatinous globule at orifice of neck. $\times 204$. Fig. 18. Mature zoosporangium from pure culture on agar showing surface exposed to air, and exit tube. $\times 239$. Fig. 19. A mature and an empty zoosporangium from culture in corn leaf; stained with chloriodide of zinc. Inner wall shows marked cellulose reaction. $\times 239$. Fig. 20. Resting sporangia in culture on regenerated cellulose film. $\times 111$. Fig. 21. Germinating resting sporangium with large central globule still evident. $\times 299$.

(Accepted for publication 2 May 1946)





NEW OR INTERESTING BRITISH FUNGI

By R. W. G. DENNIS AND E. M. WAKEFIELD

(With 26 Text-figures)

The fungi here described have been accumulating over a period of years. To all who have assisted by sending in interesting material our thanks are due.

Anthurus Archeri (Berk.) Fischer in *Jahrb. k. bot. Gart. u. bot. Mus. Berlin*, iv, 81 (1886).

This beautiful phalloid, new to this country and no doubt an introduced alien, occurred in Trewidden Gardens, near Penzance, towards the end of October 1945. The finder, Pte N. E. M. Walters, R.A.P.C., had observed the 'eggs' among rotten stumps at the base of an *Escallonia*, and recognizing that it was something unusual sent material to Kew.

The genus *Anthurus* differs from *Lysurus* in that the arms of the receptacle are not sharply distinct from the stem, and are, at least at first, united with one another at the apices. In *Lysurus* the arms are distinct from the stem and usually quite free from one another at the tips.

Anthurus Archeri is described by Cunningham (1944) as follows: Un-expanded plant obovate, to 4 cm. diameter, usually smaller, exterior furfuraceous, dingy white. Receptacle with a short, stout, hollow, usually flaring stem, attenuate and white below, slightly expanded, open and red above, to 5 cm. long, often much less, 1-2.5 cm. diameter, divided directly into five to eight orange-red, simple arms, transversely rugulose on the interior, sutured longitudinally externally, chambered, apically united when freshly expanded, but often breaking away in older plants, varying from 3 to 7 cm. in length, occasionally bifurcate at the extremities. Spore mass borne on the inner surface of the arms, foetid, olivaceous, mucilaginous. Spores elliptical, $6-7.5 \times 2-2.5 \mu$, epispore hyaline, smooth, 0.75μ thick.

The Cornish specimen had seven arms, and two pairs at least were apically united. The spores measured $5-6 \times 1.5-2 \mu$, a little smaller than the measurements given by Cunningham, but larger than those of most related phalloids. There seems little doubt that the plant can be referred to *A. Archeri*, a species widespread in Australia and Malaya.

***Psilocybe cyanescens* Wakefield, sp. nov.**

Pileus 2-4 (rarius -7.5) cm. diam., e convexo expansus, glaber, hygrophanus, viscidus, udo castaneus, sicco flavidus vel ochraceus margine striatulo, tactu cyanescens: in statu juvenile cortina delicata nivea cum stipite conjunctus. Stipes albidus, sericeo-fibrillosus, 6-8 cm. longus, 2.5-5 mm. crassus, rarius ad 10 cm. \times 7 mm., subrigidus, basi incrassatus et saepe incurvus, strigosus, tactu vel siccitate cyanescens. Lamellae e

cinnamomeo badio-fuscae, adnatae vel adnato-decurrentes, subdistantes, ad 5 mm. latae, acie pallidiore. Cheilocystidia lageniformia, circa $18-20 \times 8 \mu$. Basidia tetraspora, obclavata, supra 8μ crassa. Sporae sub lente flavo-brunneae, in massa fuscae, ellipticae, uno latere vix depressae, $10-12 \times 6-7 \times 5 \mu$, poro germinationis indistincto praeditae.

Hab. Ad terram, inter folia, ramenta, etc. in silvis, autumno, Hort. Bot. Reg. Kew.

This striking fungus has been known to the senior author for many years, and can usually be found in the autumn in the more woodland parts of Kew Gardens. It has, however, not been seen elsewhere, except once in the near neighbourhood of the Gardens, when it appeared to have originated from some rotting wood. The general appearance suggests a species near *Naucoria Myosotis*, but the spore-print is distinctly fuscous, and the decided indigo-blue colour which develops on both stem and pileus when handled distinguishes the fungus from anything which has been described in the genus *Naucoria*. A similar colour-change has been described in an American species of *Psilocybe*, *P. caerulescens* Murr., but that species is much more robust and the spores are smaller than those of the plant in question. The Kew plant in its micro- and macroscopic characters would probably be included in the genus *Deconica* W. G. Sm. by those who adopt this genus. It is retained in *Psilocybe* for the time being, until such time as a thorough revision of the purple-spored Agarics is forthcoming.*

Exobasidium Camelliae Shirai in *Bot. Mag., Tokio*, x, 51 (1896).

This fungus was described by Shirai (loc. cit.) as follows: 'Hymenium thick and white, forming a continuous layer all over the surface of the deformed organs, at first covered with a thick layer of subepidermal tissue, composed of 10 or more layers of cells, which it ruptures and breaks to a number of small pieces. Spores 4 to each basidium, oblong obovate, $14.5-17 \times 7 \mu$. This species always attacks the flower buds of *Thea* (*Camellia japonica* Nois, causing the hypertrophy and deformation of their parts. Very often the whole plant is reduced to an irregular mass of somewhat spherical form with a hollow interior, measuring 15 cm. or more in length. Common in Tokyo in May.' His Fig. 3 shows very long slender basidia with 2, 3, 3, 3, and 4 sterigmata respectively. Assuming a mean spore length of 15μ the basidia drawn were about $100-120 \mu$ long.

In June 1944 there was received at Kew an abnormal flower of *Camellia*, grown at Handcross, Sussex. Instead of the normal petals the whole flower had been transformed into a thick white, more or less globular structure. On close examination this gall was found to consist of a shallow cup with rounded and incurved edges, bearing on the outside some brown membranous fragments, apparently the remains of petals. The entire outer surface of the gall was covered with the powdery white hymenium of an *Exobasidium*, consisting of densely packed more or less cylindrical basidia gradually narrowing to their parent hyphae below. The exact base of such basidia is difficult to determine but their length appeared to be approximately $90-100 \mu$ and their greatest width $7-9 \mu$. Each bore two very short

* See p. 165.

conical sterigmata. The spores were hyaline, elongated, usually more or less comma shaped, sometimes straight, widest at the apex and narrowed towards the curved base, $15-18(-22) \times 5-7.5 \mu$, the majority about $18 \times 7 \mu$. Mature spores were sometimes one- to three-septate.

This collection appears to differ from *E. Camelliae* in having only two sterigmata, in having the outer petals almost normal and in showing no evidence of a deep-seated origin of the hymenium. In other characters there appears to be considerable agreement with the diagnosis and it is proposed to refer the Sussex collection to that species meantime. *E. Euryae* Syd. & Bull., on inflorescences of *Eurya acuminata* in Nepal, differs in host genus and hymenial characters (basidia $60 \times 5-10 \mu$, with 2-4 sterigmata, spores $14-17 \times 4 \mu$) and the other species of *Exobasidium* described on Ternstroemiaceae appear to be confined to the leaves.

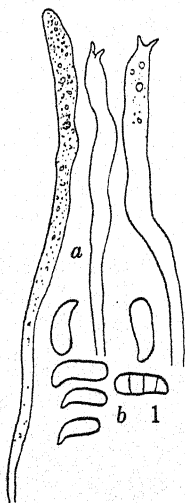


Fig. 1. *Exobasidium Camelliae* Shirai. a, basidia; b, spores ($\times 375$).

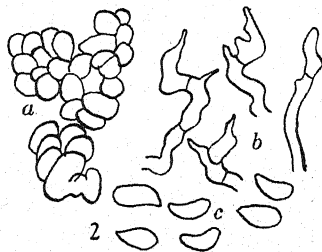


Fig. 2. *Herpobasidium filicinum* (Rostr.) Lind on *Dryopteris Filix-mas*. a, mycelium from three cells of host leaf; b, four basidia; c, six shed basidiospores (all $\times 300$).

Herpobasidium filicinum (Rostrup) Lind in *Ark. Bot.* VII (8), 7 (1908).

Syn.: *Gloeosporium filicinum* Rostrup in *Thuem. Myc. Univ.* no. 2083 (1881).

Exobasidium Brevieri Boudier in *Bull. Soc. Mycol. Fr.* XVI, 15 (1900).

The fructifications of this fungus form white mould-like spots, mostly about 1 mm. across but sometimes confluent, on the lower surface of *Dryopteris* and *Polypodium* leaves. Corresponding small brownish angular spots appear on the upper surface of affected fronds. Infected host cells contain conspicuous coiled haustoria typical of the genus. The superficial mould consists of fine, hyaline, septate hyphae bearing terminal, two-celled basidia with stout sterigmata. The basidiospores are oval to oblong-apiculate, thin-walled, $18-24 \times 6-9 \mu$, sometimes germinating *in situ* to produce secondary spores. These spore dimensions are a little greater

than those cited by Lind for his collections on *Dryopteris Filix-mas*, viz. $10-18 \times 5-8 \mu$, basidia $40-50 \times 9 \mu$, sterigmata up to 17μ long.

British collections of this species are:

On *Dryopteris Filix-mas*, Breidden Hill, Montgomeryshire, 9 July 1942, N. Y. Sandwith; Harpenden, Hertfordshire, June 1931, A. Smith.

On *Polypodium Phegopteris*, Scotland, 25 June 1932, K. W. Braid.

It has been reported elsewhere on *Polypodium Dryopteris* and *Cystopteris montana*. According to Lind (op. cit.) Boudier's citation of *Athyrium Filix-femina* as a host of this fungus was an error. The cytology of this species has been fully investigated by Jackson (1935).

Puccinia commutata Sydow, *Monographia Uredinearum*, 1, 201 (1904).

P. Valerianae auctt. non Carest.

Aecidia developed on the lower surface of leaves, on petioles and on stems; on the leaves forming rounded or irregular groups; on stems arranged in more or less elongated series, seated on pale spots. Aecidiospores polygonal, finely warted, orange, $14-19 \mu$ in diameter. Teleutospores hypophyllous, or on petioles or stems; on the leaves scattered or crowded, sometimes confluent, minute; on the stems generally densely crowded and confluent, forming more or less elongated pustules, pulverulent, dark brown. Teleutospores irregular, oblong, subfusoid or subclavate, moderately thickened at the apex, rounded or somewhat conical, attenuated with little or no constriction at the septum, narrowed at the base, rarely rounded, smooth, chestnut brown, $40-60 \times 20-35 \mu$: pedicel rather long, hyaline, deciduous.

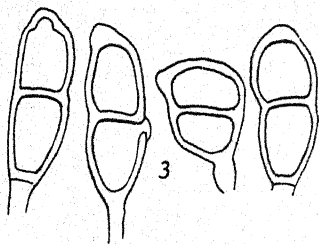


Fig. 3. *Puccinia commutata* Syd., teleutospores, apices swollen with potassium hydroxide ($\times 375$).

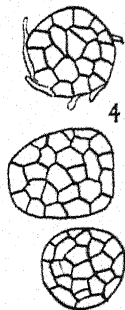


Fig. 4. *Tolyposporium Montiae* Rostr. from *Montia fontana*. Spore-balls ($\times c. 250$).

On leaves and petioles of *Valeriana officinalis*, Loch Scridain, Mull, Argyllshire. Coll. T. A. Russell, 14 August 1928.

The aecidia and teleutospores occur intermixed, and on the petiole cause a certain amount of swelling and distortion. The species is widespread in Europe, but has frequently been referred to *Puccinia Valerianae* Carest., which is confined to *Valeriana celtica* and differs in the characters of the teleutospore.

Tolyposporium Montiae Rostrup (Vejledn. i den danske Flora, II, 31, 1904).

Sorosporium Montiae Rostrup in Bot. Tidskr. xx, 129 (1896).

Sori black; spore-balls subglobose or irregular, black, opaque, 45–60 μ in diameter, consisting of numerous spores. Spores irregular, polygonal, smooth, fuscous, not easily separating, 5–8 μ in diameter or $10 \times 5 \mu$.

On leaves and stems of *Montia fontana*, West Kilbride, Ayrshire; D. A. Boyd, 3 June 1927.

The spore measurements given above are taken from the specimen in hand. In the original description the spore-balls are said to be somewhat larger, namely 60–80 μ . They are scattered throughout the lamina of the lower leaves, making them appear black to the naked eye and black-punctate under a lens. In the material to hand they are concentrated towards the leaf tips and scattered more sparsely towards their bases. Sori have not been seen on the stems.

Ustilago echinata Schroeter, Abh. d. Schles. Ges. naturw. Abt., 1869, 4 (1871).

The species is omitted from the list of British smuts though possibly represented by Masee's reference to *U. longissima* (Schlecht.) Meyer on *Phalaris arundinacea* (Sampson, 1940). A leaf smut of *Digraphis arundinacea* presumably referable to *Ustilago echinata*, with spores $10\text{--}13 \times 7\text{--}11 \mu$ and very variable in shape, was collected in July and August 1943 at Bridge of Dun, Angus, and Castle Douglas, Kirkcudbrightshire. As this species seems to be doubtfully distinct from *U. striiformis* (Westend.) Niessl it will not be described more fully here. Fischer & Hirschhorn (1945), however, believe *U. echinata* can be separated from *U. striiformis* by its somewhat larger, rougher spores. At both localities other plants of this host in the same reed beds bore the head smut *Tilletia Menieri* Har. & Pat., already known from Ireland but not hitherto from Great Britain.

Lilliputia insignis (Winter) n.comb.

On 29 March 1938 specimens of a fungus on mushroom compost from Uxbridge, Middlesex, were received at Kew from Mr W. Buddin. The fructifications were spherical, glabrous, whitish to cream coloured, approximately 500 μ in diameter and scattered singly over the surface of the compost. Microtome sections showed them to have a stout wall, some 80 to 90 μ thick, composed of ten to twelve layers of cells, differentiated into an outer belt, three to four cells wide, of empty cells, and an inner zone, seven to eight cells wide, densely packed with oil globules. The centre of the ascocarp consisted of thin-walled tissue in which were embedded numerous asci, each with eight spherical yellow ascospores. The latter measured 11–13 μ in diameter with a large central oil globule and a wall about 1 μ thick, densely studded with short blunt warts. These measurements were made on spores within the asci.

Except for spore size this agrees precisely with the diagnosis and figures of *L. Gaillardii* described from humus in a glasshouse at Angers, France, April 1900. According to Boudier and Patouillard (1900) this fungus had spores 22–24 μ in diameter. Fortunately, type material of the species was

preserved by Patouillard and is now in the Farlow Herbarium. Dr Linder has kindly re-examined this and reports as follows: 'I have studied the type of *Lilliputia Gaillardii* Boud. & Pat. and find that the spores measure $13.2-18.2 \times 18.2-24.5 \mu$. These spores are for the most part still within the ascus and tend to be somewhat compressed and therefore angular. In the few cases where the spores had been freed from the ascus the spores are perfectly globose, as stated by Patouillard.' It appears, therefore, that the spores of this fungus vary greatly in size and that the Angers and Uxbridge collections represent the same species. It is also the same as the fungus distributed under the name *Gliocladium penicilloides* Corda as number 120 of Reliquiae Farlowianae. This had been collected on decaying seaweeds at Kittery Pt., Maine, U.S.A., on 3 July 1918 by Thaxter, who cited as synonyms *Penicillium insigne* Bainier, *Licopenicillium insigne* Brefeld and *Lilliputia Gaillardii* Boud. & Pat. (Thaxter, 1922). Thom (1930), however,

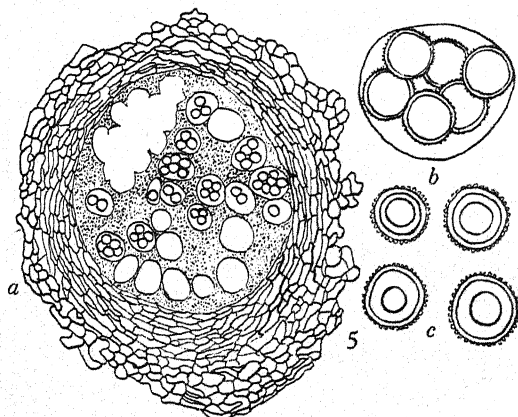


Fig. 5. *Lilliputia insigne* (Wint.) n.comb. a, microtome section of ascocarp from Middlesex ($\times 100$); b, above: single ascus; below: two ascospores in optical section, from Middlesex material ($\times 750$); c, ascospores from Rabenhorst, *Fungi Europaei*, no. 1732 ($\times 750$).

regarded *Penicillium insigne* as probably distinct from *Gliocladium penicilloides* and the applicability of the latter name is also dubious as will be shown below.

Previously Winter (1873) had obtained an ascomycete on goose dung in Germany which he regarded as the perfect state of *G. penicilloides*. No description was published but material with adequate figures of asci and ascospores was distributed by Rabenhorst as *Fungi Europaei*, no. 1732, under the name *Eurotium insigne* G. Winter n.sp. (an n.g.?). Examination of Winter's material at Kew shows it to correspond closely with the present fungus except that the ascocarps are now a deep yellow-brown and the outermost cells appear deep yellow in section. The ascospores measure $13-15 \mu$ across. Masee and Salmon (1901), who obtained *E. insigne* on dung of kangaroo, *Ovis burrhel*, fowl, and horse, at Kew described it as having the perithecia clear white when young, then pallid, becoming yellowish and finally rusty brown, with ascospores globose, $17-20 \mu$ across.

The colour difference mentioned above is thus clearly not significant and depends probably on the mode of preservation. Winter's collection has been dried for over 70 years, the Uxbridge material has been kept in formalin.

These and other records of *Eurotium insigne* have been discussed by Petch (1939) who concluded that the imperfect fungus associated with it is not *Gliocladium penicilloides*, which he ascribes to *Hypomyces aureo-nitens* Tul., but *Gliocladium macropodinum* Marshal. The name adopted by Thaxter is thus inappropriate both on the ground of misidentification and of applicability only to an imperfect state. Though association with a *Gliocladium* was not noted in the Uxbridge material nor mentioned by Boudier and Patouillard, it appears at present highly probable that all the collections cited above are the same species. Owing to the very characteristic and distinctive ascospores it seems inadvisable to refer this fungus to *Eurotium*; it would appear preferable to retain the genus *Lilliputia*, with the new combination *L. insigne*. Boudier and Patouillard ascribed *Lilliputia* to the Tuberaceae, which is clearly inadmissible; Clements and Shear (1931) transferred it to Gymnoascaceae, in which it is equally out of place. Pending detailed studies of the developing ascocarp it seems to fall most naturally in Eurotiaceae, near *Eurotium*, from which it is distinguished by the spherical, warted ascospores.

Sphaerulina myrtillina Sacc. & Fautr. in *Bull. Soc. Mycol. Fr.* xvi, 21 (1900).

Spots amphigenous, reddish purple, rounded, 1–1.5 mm. in diameter, often confluent, eventually paler in the centre. Perithecia formed in the paler dead tissue, few, scattered, black, erumpent, 100–120 μ in diameter. Asci large, oblong or somewhat saccate, sessile obtuse, about 200 \times 50–60 μ , eight-spored, paraphyses lacking. Spores more or less distichous, oblong, obtuse at both ends, finally tri-septate, slightly constricted at the median septum, hyaline, 45–50 \times 15–18 μ .

On living stems of *Vaccinium Myrtillus*, Ben Ledi, Perthshire; W. B. Turrill, 1 September 1928.

The species is remarkable for the very large asci and spores. It has been removed by von Hoehnel to his genus *Pseudosphaeria*. As, however, doubt has been cast on the validity of the whole conception of the group Pseudosphaeriaceae, it seems at present best to retain the original name.

Sordaria caudata (Curr.) Sacc., *Syll.* i, 236.

Sphaeria caudata Currey in *Trans. Linn. Soc.* xxii, 320 (1859).

A small gathering of this apparently rare species was obtained from very soft, rotten wood in Rothiemurchus Forest, during the Aviemore Foray, 1927. Comparison with Currey's figure and with his specimen in the Kew Herbarium leaves no doubt as to its identity.

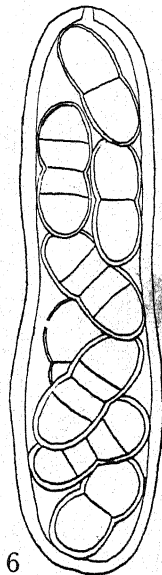


Fig. 6. *Sphaerulina myrtillina* Sacc. & Fautr. Ascus ($\times 375$).

The perithecia are conical, very thin-walled and frequently collapsed, with the base sunken in the rotten wood. The spores, at first hyaline and cylindrical, at length develop a dark brown head, $17-20 \times 9-10 \mu$, and a long, pale or hyaline tail, $30-32 \times 4-5 \mu$. There is in addition a small, hyaline, pointed apical appendage, which is not shown in Currey's figure, but is present in his specimen and is also depicted by Berkeley and Broome (1871, tab. 21, fig. 25).

Leptospora caudata Fckl., *Symb. Mycol.* p. 144 (1869).

Perithecia superficial, gregarious, rather large, ovate, obtuse, rugulose, black, covered with short, multiseptate, dark, branched hairs; ostioles obtuse, perforate, subglabrous; asci long-stipitate, elongated, eight-spored, spore-bearing portion 128μ long, 12μ in diameter; spores oblong, obtuse at the apex, base oblique, acuminate-caudate, unseptate, multiguttulate, hyaline, $32 \times 6 \mu$.

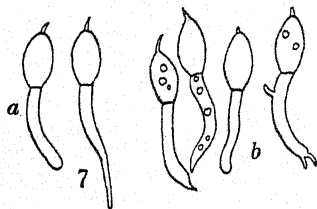


Fig. 7. *Sordaria caudata* Currey. *a*, two ascospores from type specimen; *b*, four ascospores from Aviemore specimen ($\times 375$).

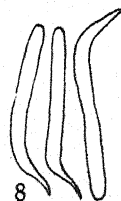


Fig. 8. *Leptospora caudata* Fckl., from Tintern. Ascospores ($\times 600$).

The above is a translation of Fuckel's description. At the Tintern Spring Foray there was collected a Pyrenomycete which apparently belongs to the above species. Unfortunately, the material is so scanty that it is impossible to say whether it agrees with the description in all details. The perithecia in general appearance suggest *L. spermoides*, but are more rough. A covering of branched hairs is not now present, but as many of the perithecia are empty it is obvious that the material is old, and such a character may well be evanescent. The characteristic feature lies in the peculiar spores. In the Tintern specimen they are exactly as described by Fuckel, hyaline, unseptate, with a knee-like bend at the base terminating in a slender 'tail'. They measure $35-40 \times 4 \mu$. Asci not seen.

On dead larch twig, Tintern, May 1925.

Under the manuscript name of '*Sphaeria falcispora* Broome' there is in the Kew Herbarium a specimen with similarly shaped spores. The perithecia in this material are at first distinctly hairy, becoming smooth with age, and in this respect the fungus would agree with Fuckel's description. The spores, however, are longer even than those of the Tintern specimen, being $67-70 \times 5 \mu$.

Nectriella Lophocoleae Massal. in *Mem. dell' Acc. delle Sci. Med. e Nat. di Ferrara*, 5 Magg. 1895.

This interesting fungus was described from living leaves of the liverwort

Lophocolea cuspidata near Massa Conora, Italy. The diagnosis runs as follows: Perithecia superficial, solitary to subaggregated, pale orange, ovoid, seated on a light coloured filamentous subiculum, 120–150 μ in diameter, covered with bristly setae, setae continuous, thick-walled, simple, 8–10 μ thick, about half the length of the perithecium, ostiole apical, indistinct, wall membranaceous; asci cylindrical clavate, rather long pedicellate-stipitate, fertile portion 70–110 \times 16–22 μ . Spores eight, ellipsoidal, 22–30 \times 10–14 μ , hyaline, rounded at the ends, guttulate; paraphyses numerous, filiform, 1–1.5 μ thick, as long as the asci, sometimes branched.

A few specimens of this species were collected by Miss M. Buchanan on *L. bidentata* at Dargle near Dublin, Éire, in March 1929, and sent to Kew by Miss M. C. Knowles. The perithecia were reddish brown, covered with subhyaline blunt setae, up to



Fig. 9. *Sphaeria falcispora* Broome. Two ascospores, from specimen in Herb. Kew. ($\times 600$).

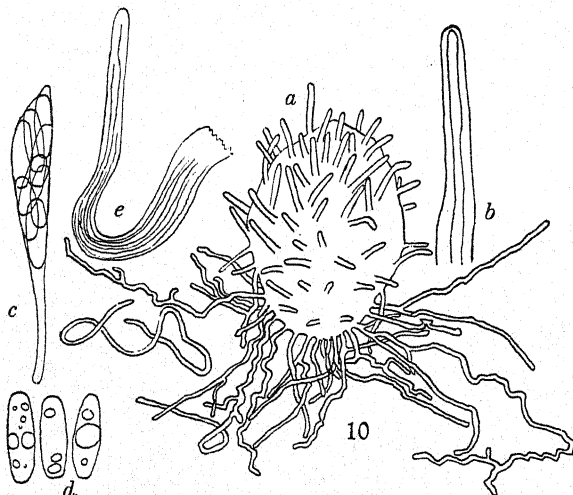


Fig. 10. *Nectriella Lophocoleae* Massal. a, perithecium ($\times 60$); b, seta from same perithecium ($\times 300$); c, undischarged ascus; d, three discharged ascospores ($\times 300$); e, empty ascus ($\times 300$).

c. 140 μ long and 8–9 μ thick. Asci and paraphyses were freely liberated from the apex of the perithecium by slight pressure on the cover slip and the spores appeared to emerge from the asci in groups of eight, as though still enclosed in a fine membrane. They measured 34–40 \times 12–13 μ . In Petch's (1938) classification this species appears to be a *Pseudonectria*.

Rhytisma symmetricum J. Müller in *Pringsh. Jahrb. f. wiss. Bot.* xxv, 622 (1893).

Spots numerous on each leaf, black, irregular in shape, smaller than in *R. salicinum*. Stromata extending right through the leaf and equally prominent on either side, 1–3 mm. in diameter. Apothecia amphigenous. Asci 135–162 \times 12–19 μ ; spores filiform, 30–108 μ long. Paraphyses short, slightly enlarged above.

The measurements of asci and spores given are from the original description. The material sent was immature, but the species is easily distinguished from *R. salicinum* by the numerous small spots on the leaf, and especially by the symmetrical development of the stromata on both sides of the leaf.

On leaves of *Salix*, Great Yarmouth. Received from Dr G. H. Pethybridge, October 1926.

A NEW *SCLEROTINIA* ON *GLADIOLUS*

In the autumn of 1938 Mr W. Buddin was working on the diseases of *Gladiolus* corms, and isolated what appeared to be two different species of *Botrytis*. One, the more common, was of the *B. cinerea* type, with sclerotia, but these not very abundant. The other, with more narrowly elliptical conidia, produced abundant sclerotia aggregated in large masses, which in appearance suggested those of *B. convoluta* Drayton, described from *Iris*. They were, however, not truly convolute. Using the technique described by Drayton (1934) for *Sclerotinia Gladioli*, Mr Buddin was successful in producing by spermatization the apothecia of this form, and brought material to Kew in the early part of 1939. As might have been expected, the apothecia and spores do not differ greatly from those of other species included by Whetzel in his genus *Botryotinia* (i.e. species of *Sclerotinia* having a conidial stage of the *Botrytis cinerea* type), but the distinctive characters of the conidia and sclerotia distinguish this fungus from any other so far known on *Gladiolus*, and appear to warrant the establishment of a new species. Circumstances have prevented the writing up of the whole of the work, and it seems advisable now to publish the diagnosis of the new species, so that it may be available for other workers in the same field. The authors have given the specific epithet in honour of Dr F. L. Drayton of Ottawa, whose unfailing interest and generosity in connexion with the work has been of much encouragement.

***Sclerotinia Draytoni* Buddin & Wakef., sp. nov.**

Sclerotia atra, laevia, applanata demum convexa (lenticularia), circa 8-12 × 3-7 mm., in massis irregularibus usque ad 2.5 cm. longis agglutinata, intus albida, ex hyphis hyalinis crasse tunicatis arcte intertextis composita. Micro- et macroconidia adsunt. Microconidia globosa, 2-2.5 μ diam. Macroconidiophora e sclerotiis oriunda, erecta, deorsum fusca, 12-15 μ diam., sursum hyalina, ramosa. Rami 2-3, obtusi, interdum furcati, plerumque sursum capitato-incrassati, sterigmatibus minutis deciduis instructi. Conidia hyalina vel pallide fusca, cylindrico-ellipsoidea vel anguste ovata, 8-16 × 5-7.5 μ. Apothecia longe stipitata, singulatim vel gregatim e sclerotiis enascentia, primo infundibuliformia margine incurvata, dein umbilicato-discoidea, denique leviter convexa, margine reflexa. Hymenium 2.5-5 mm. diametro, ex argillaceo fulvo-olivaceum margine primo brunnea, demum pallidiore, demum concolor. Stipes tenuis, elongatus, 10-13 mm. longus, flexuosus, sursum incrassatus, hymenio concolor, basim versus fuscescens, plerumque glaber sed aliquando ob condiciones humidus pilosus. Asci cylindrici, octospori, 140-190 μ longi, 7.5-9 (-10) μ crassi,

pars sporifera circa 80μ longa. Ascosporae monostichae, anguste ellipsoideae vel subfusiformes, hyalinae, saepe biguttulatae, $12-17 \times 6-8\mu$ (medio $15 \times 6\mu$). Paraphyses filiformes, apice clavato incrassatae.

Hab. In caulibus Gladioli sp. cult., parasitica.

Sclerotinia hirtella Boudier, *Icon. Mycol.* III, tab. 471 (1907).

Two specimens which appear referable to this species were found on 29 May 1945 arising from sclerotia attached to fallen bud scales of some deciduous tree lying in a damp situation under a clump of rushes on Leith

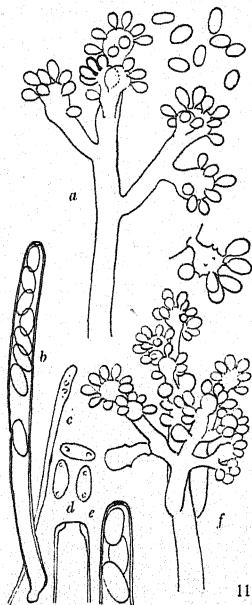


Fig. 11. *Sclerotinia Draytoni* Buddin & Wakef. a, conidiophore ($\times 400$) and conidia ($\times 250$); b, ascus; c, paraphysis; d, three spores ($\times c. 325$); e, apices of two asci ($\times 500$); f, conidiophore ($\times 250$).

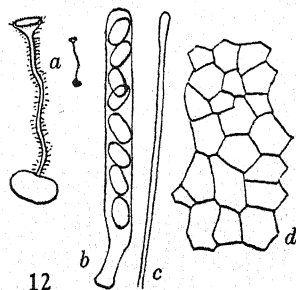


Fig. 12. *Sclerotinia hirtella* Boud. a, sclerotium and ascocarp, nat. size and $\times 3.75$; b, ascus; c, paraphysis; d, superficial cells of sclerotium ($\times 450$).

Hill, Surrey. Boudier's collection was on rotting sweet chestnut twigs but it is impossible to say whether this holds for the Surrey material or not, though sweet chestnut is plentiful in the vicinity. Boudier's diagnosis is as follows: Apothecia minute, slender, stipitate, 5-10 mm. high, entirely pale ochraceous-cinereous, hairy, arising from a small, black oblong sclerotium. Receptacle at first cup-shaped, ultimately flattened, externally covered, like the stipe, with long, flexuous septate, pointed, colourless hairs, $90-110\mu$ long $\times 4\mu$ thick, and simple or bifid. Hymenium smooth and concolourous, paraphyses linear, colourless, septate, slightly thickened to about 3μ at the tip; asci cylindrical, very slightly attenuated at the base, eight-spored, inoperculate, $90-115\mu$ long by $7-8\mu$ wide; spores oval-oblong, colourless, non-guttulate, $7-9 \times 4-5\mu$.

Ascotremella faginea (Peck) Seaver in *Mycologia*, xxii, 53 (1930).

Haematomyces fagineus Peck in *Ann. Rep. N.Y. State Mus.* xliii, 33, 1890.

In August 1944 Mr A. A. Pearson sent to Kew a fungus which proved to be of considerable interest. At first sight it suggested the conidial stage of *Coryne sarcoides*, but was more cerebriform and of a rather duller colour. On microscopic examination it was found to be an Ascomycete (Discomycete) but without distinct cup-like apothecia, and the spores were quite different from those of *Coryne*. The only species found in literature to which it can be referred is *Ascotremella faginea* (Peck) Seaver. In the article referred to in *Mycologia*, Seaver has shown that the generic name *Haematomyces* cannot be used for this fungus. The original species on which the genus *Haematomyces* was founded has been shown to be a resinous exudation, not a fungus at all. Later authors have included in the genus, Ascomycetes which are not congeneric, and it would therefore be best to abandon the name *Haematomyces* altogether. For these plants which are tremelloid in habit but have asci with hyaline unseptate spores, Seaver has proposed the new name *Ascotremella* designating *A. faginea* as the type species.

Mr Pearson's plant agrees on the whole very well with the description given by Seaver, which was apparently compiled from dried material and photographs. The colour, however, of the British plant is rather more purplish than is suggested by the description 'raisin-coloured', the asci are longer and the spores tend to be subdistichous rather than clearly monostichous (the description says 'usually one-seriate'). The following description was drawn up from the fresh plant:

Ascoma tremelloid, cerebriform, gelatinous, smooth, dull reddish purple in colour when fresh and about 4 cm. in diameter, but shrinking when dry to a hard, horny mass less than half that size. Asci covering the whole lobed outer surface, cylindrical, up to 95μ long, 7μ broad above, inoperculate, spores occupying less than half the total length, subdistichous, eight in number. Spores elliptical, hyaline, with two oil-drops, thin-walled, $(6-8-10 \times 4\mu)$. Paraphyses slender, hyaline, slightly thickened towards the apex. Hyphae of hypothecium $2-6\mu$, embedded in a gelatinous matrix.

On dead wood, West Dean Wood, near Goodwood, Sussex. Coll. A. A. Pearson, August 1944.

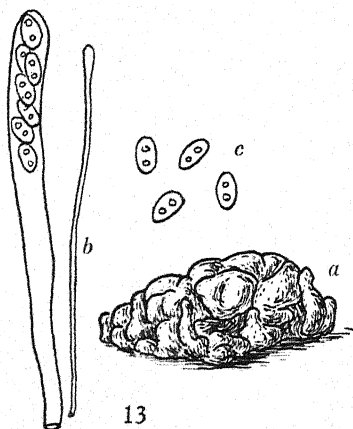


Fig. 13. *Ascotremella faginea* (Peck) Seaver. a, Ascoma (nat. size); b, ascus and paraphysis ($\times 600$); c, spores ($\times 600$).

Protomyces inundatus Dangeard in *Le Botaniste*, ix, 274 (1906).

After pointing out that on most hosts the sporangia of *Protomyces macrosporus* Ung. germinate by rupture of the episporium, followed by emergence of the contents still surrounded by endospore and mesospore before sporu-

lation, Dangeard remarked that in the race on *Helosciadium nodiflorum* the spores form in the interior of the unbroken sporangium. He continued: 'Must we found a new species, characterized by this mode of germination? We think not, and it is simpler, we believe, to attribute this difference in mode of germination to the difference in habitat. When the parasite lives on a terrestrial umbelliferous host the chlamydospores extrude their sporangia to the exterior, when, as here, it is on an aquatic host, germination occurs under different conditions in that the host is totally or partly immersed. If, however, one wishes to establish a new species we propose the name *Protomyces inundatus*.' von Büren (1922) showed experimentally that *P. inundatus* on *Helosciadium nodiflorum* could not be transferred experimentally to *Aegopodium Podagraria*, *Sium erectum*, *Apium graveolens* or *Helosciadium inundatum*. In view of this close biological specialization he accepts it as a species distinct from *Protomyces macrosporus*. He retains it, however, in *Protomyces*, in contrast to his earlier view (v. Büren, 1915) that the fungus on *Helosciadium nodiflorum* was a *Taphridium*. The name *Protomyces inundatus* Dangeard was accordingly compiled in Saccardo, vol. xxvi, but no formal diagnosis of the species has been traced.

P. inundatus is not uncommon on *Helosciadium nodiflorum* in Britain. Collections exist at Kew from Reading, 6 July 1943, L. Hawker; Farlington Marshes near Plymouth, 24 August 1945, J. Staley; Conway, 27 June 1927, W. B. Grove; Cefn, Beaumaris, Anglesey, 29 July 1927, P. G. M. Rhodes; Wirral, 22 December 1912, J. W. Ellis; Scarborough, 7 August 1911, T. B. Roe. A collection from Shrewsbury, July 1883, which appears to be the same fungus, is filed under the manuscript name *Protomyces Helosciadii* Phil.

Pseudoperonospora Erodii (Fuckel) G. W. Wilson in *Mycologia*, vi, 194 (1914).

Syn. *Peronospora Erodii* Fuckel, Fungi Rhenani, no. 2102 (1868).

Plasmopara Erodii (Fuckel) D. Sacc. *Mycoth. Ital.* no. 890.

This rather inconspicuous downy mildew was found associated with small brown lesions up to 5 mm. long on living leaves of *Erodium cicutarium*, near Dorking, Surrey, 3 June 1945. The conidiophores form dense, greyish violet clusters on the lower surface of the leaf, about 100–300 μ long, 4–6 times dichotomously branched, with the main stem occupying from one-half to two-thirds of the total length, 8–10 μ thick, swelling to 13 μ at the base, and terminal branches 5–10 μ long, slightly curved. Conidia are yellow-brown and vary from almost globose bodies 16–23 μ across to ovoid ones 23–35 \times 18–24 μ .

Peronospora Gei Sydow in Gaumann, *Beitr. Krypt. Flora der Schweiz*, v, Heft 4, 291 (1923).

On 13 June 1945 Mr H. K. Airy Shaw collected leaves of cultivated *Geum*, varieties Mrs Bradshaw and Lady Stratheden, in the Rectory garden at Daglingworth, Glos., heavily infected by *P. Gei*. Many plants showed the lower surface of the larger leaves almost completely covered with a greyish coating of conidiophores and conidia; in others sections of the

lamina delimited by the main lateral veins were similarly affected. Corresponding chlorotic patches on the upper surface of the leaves rendered the diseased plants extremely conspicuous. It is the more remarkable that this destructive disease seems to have hitherto remained unrecorded in Britain. The diagnosis of *P. Gei* follows: Conidiophores densely crowded, in a greyish white felt, covering parts of the lower surface of the leaf;

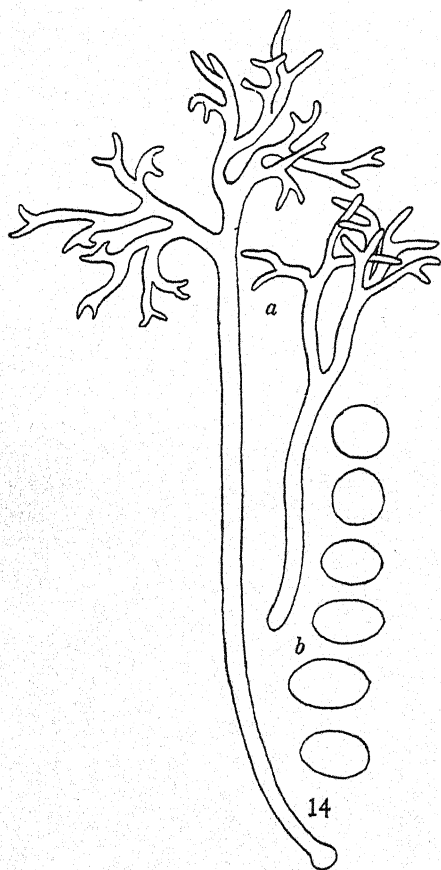


Fig. 14. *Pseudoperonospora Erodii* (Fckl.) G. W. Wilson. *a*, two sporophores; *b*, six conidia ($\times 300$).

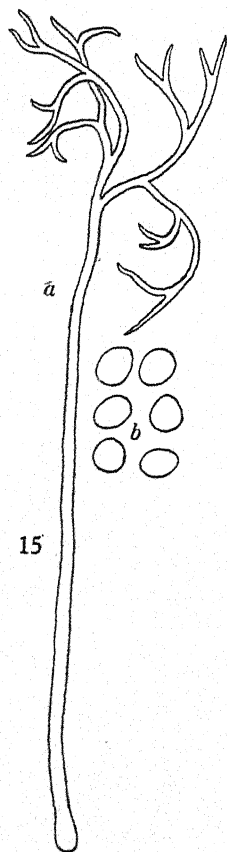


Fig. 15. *Peronospora Gei* Syd. on cultivated *Geum*. *a*, sporophore; *b*, six conidia ($\times 300$).

commonly extruded in groups from the stomata, 200–500 μ long, the main axis forming one-half to two-thirds of the total length, 4–8 μ thick, base slightly swollen, four to six times dichotomously branched, branches spreading, undulating; terminal branches 10–30 μ long, much curved. Conidia light brown, broadly ellipsoidal, 8–24, mostly 15–19 μ long, 8–21, mostly 13–18 μ broad; mean size $17.09 \times 15.26 \mu$. Oospores unknown. The type collection was apparently on *Geum album* from North America,

but Gaumann regarded forms on *G. rivale* and *G. urbanum* as belonging to the same species, probably also those on *G. coccineum* and *G. macrophyllum*. *G. urbanum* in the vicinity of the diseased garden plants at Daglingworth remained healthy.

TWO FUNGI OF APPLE LEAF SPOTS

In September 1944 we received from Dr E. Gray of the north of Scotland Agricultural College, Aberdeen, three apple leaves from Achnashellach, Ross-shire, marked by more or less circular, whitish spots of dead tissue, between 2 and 6 mm. in diameter. Each spot was sharply defined by a narrow dark brown line and bore on its upper surface numerous (up to 90) minute black pycnidia, just visible to the unaided eye. Examination showed that though the pycnidia appeared uniform externally, even from a single spot some yielded hyaline, non-septate, somewhat fusiform and usually biguttulate spores measuring $2.5-6 \times 2-4 \mu$, mostly about $5 \times 3 \mu$, while others contained very pale brown, elliptical, non-guttulate spores measuring $4-5 \times 2-3 \mu$. The former was tentatively identified with *Phyllosticta Mali* Prill. & Del., the latter with *Coniothyrium tirolense* Bub. not hitherto reported from Britain. Cultures from spores of the *Coniothyrium* type yielded a thin brown mycelial mat with scattered clusters of black pycnidia which contained typical *Coniothyrium* spores $3-5 \times 2-2.5 \mu$ with a mean size of $4.3 \times 2.8 \mu$. No lesions were induced by this fungus on Doon Star potato tubers, but on apple fruit it caused small, round, superficial, blackish lesions and on tomato fruit it gave rise to a rather slow rot which attained a diameter of 14 mm. in 8 days at room temperature. All inoculations were made by inserting a fragment of malt agar culture into a minute slit in the surface of fruit or tuber concerned.

According to Wollenweber and Hochapfel (1937) *C. tirolense* has persistently light brown pycnosporos of mean size $5.6 \times 3 \mu$, causes hard, leathery, black spots on apple fruit, and is able to rot tomatoes and pears. The identification of the Scottish fungus with that species was therefore upheld by the inoculation experiments described above. There is no material of *C. tirolense* under that name at Kew but a collection of *C. piricola* Potebnia, distributed by Savulescu as Herb. Mycolog. Romanicum, no. 1171 and preserved in the herbarium of the Imperial Mycological Institute, yielded similar spores $3-7.5 \times 2-3.5 \mu$, mean size $5.7 \times 2.9 \mu$. It thus agreed with Wollenweber and Hochapfel's conception of *C. tirolense*, of which they regard *C. piricola* as a synonym. A further specimen in the same herbarium, collected on 'crab' apple at Indian Head, Saskatchewan, which had spores $4-6 \times 2.5-3.5 \mu$, mean size 5.1×3.0 , had been determined by Dr G. R. Bisby as *C. pirinum* (Sacc.) Sheldon. The spots appeared identical on the leaves of all these collections and though the spores of the Scottish material are a shade smaller than the others there seems no reason to doubt that all three represent the same species.

Owing to its presence on the same small spots it was natural to suppose that *Phyllosticta Mali* might be an early stage in the development of this fungus, but, owing to the difference in shape as well as colour of the spores, it seemed desirable to check this attribution by artificial cultures. Single

pycnidia of the *Phyllosticta* and of the *Coniothyrium* were therefore cut out of the spots and crushed in sterile water and cultures were started from the resultant spore suspensions on maize meal agar on 14 April 1945. The spores of both fungi were still freely viable although the leaves had been stored dry in a herbarium capsule for seven months. Eleven isolations from the same pycnidium of *Phyllosticta Mali* yielded apparently identical cultures of a rather quick-growing fungus which produced numerous, scattered, yellow-brown pycnidia within four days at room temperature. After a fortnight's growth there were still no aerial hyphae and no dark colour in the submerged mycelium so that the pycnidia formed almost the only features visible when the plate was held up to the light. On malt agar slants, there was a profuse development of short, dense, whitish to pinkish brown aerial mycelium passing into a greenish brown zone near the top of the slant. Eight isolations from a single pycnidium of the *Coniothyrium* yielded a rather slower growing fungus which produced a dense clump of aerial mycelium round the points of inoculation. This mat, at first white, rapidly turned grey and after a fortnight's growth each isolate consisted of a central dome of grey aerial hyphae surrounded by a more or less distinct, short, grey-brown mat, succeeded by a broad marginal belt free from aerial mycelium. Black pycnidia were scattered sparsely, singly and in small groups, over the central portion of each mat. Mycelial growth on malt agar slants was much more copious and scarcely distinguishable from that of the *Phyllosticta*, the aerial mat was, however, darker brown than in the latter fungus and pycnidia were far less abundant.

The *Phyllosticta* pycnidia produced in culture were approximately 200–250 μ in diameter, with one or often two well-defined ostioles, and bore a pink spore exudate consisting of hyaline spores, similar in shape to those produced on the host but less distinctly guttulate, and measuring $4\text{--}6 \times 2\text{--}5\text{--}3 \mu$, mean size $4\text{--}5 \times 2\text{--}7 \mu$. The *Coniothyrium* pycnidia were similar in size but from the first yielded yellow-brown spores $3\text{--}4 \times 2\text{--}5\text{--}3 \mu$, mean size $3\text{--}2 \times 2\text{--}7 \mu$. The two fungi were therefore clearly distinct and did not belong to the same life cycle.

Reference to the literature shows that similar observations were made on apple leaf-spot fungi by Crabill (1912) in Virginia in 1911. The *Phyllosticta* described above agrees fairly well with his strain 2, identified as *P. pirina* Sacc. while the *Coniothyrium* seems to be his conception of *C. pirinum* Sheldon. The question therefore arises, what are the correct names of these two fungi?

The name *Phyllosticta pirina* was applied by Saccardo (1878) to a pycnidial fungus of pear and apple leaves described as having ovoid to ellipsoid hyaline spores $4\text{--}5 \times 2\text{--}2\text{--}5 \mu$. The type is apparently preserved in Saccardo's herbarium at Padua (Gola, 1930) and without reference to it, it is scarcely possible to be certain what his fungus was, but the absence of any reference to guttules and the description of spore shape suggests an early state of the *Coniothyrium* rather than the *Phyllosticta* described above. Sheldon (1907), in West Virginia, collected a *Coniothyrium* on apple and quince leaves which he identified with Saccardo's fungus, and accordingly renamed the latter *C. pirina* (Sacc.) Sheldon. 'Specimens of the fungus on

apple and quince leaves were submitted', to Saccardo, 'for determination and comparison with type specimens of *Phyllosticta pirina* Sacc.' Presumably Saccardo accepted the identification though Sheldon does not precisely say so. Meanwhile Bubák (in Bubák and Kabát, 1904) had described *Coniothyrium tirolense* as a parasite of living pear leaves in Tyrol, in association with *Phyllosticta tirolensis* Bub. Three years later Potebnia (1907) published the name *Coniothyrium piricola* for a similar fungus on apple leaves in Russia, associated with a *Phyllosticta* identified as *P. Briardi* Sacc. Wollenweber and Hochapfel (1937) reduced *Coniothyrium piricola* to a synonym of *C. tirolense* and, assuming Sheldon's identification to be correct, there seems every reason to regard both names as synonyms of *C. pirina*.

When Crabill demonstrated the occurrence of a *Phyllosticta* distinct from *Coniothyrium pirinum* on apple leaf spots in Virginia he adopted the names *Phyllosticta pirina* Sacc. and *Coniothyrium pirina* Sheldon but as Sheldon's name originated in a transfer of Saccardo's specific epithet from *Phyllosticta* to *Coniothyrium* and the same type collection can hardly be held to be the type of two different species these names would seem to be inadmissible. Meanwhile, Prillieux and Delacroix (1890) had described a *Phyllosticta Mali* on apple leaves at Lorient, France, which seems clearly to be the *Phyllosticta* both of Crabill and of the Scottish material. Though their meagre diagnosis merely refers to 'sporulis ovoideis 6·5-8·5 × 4-4·5 μ' their figure distinctly shows rather fusiform biguttulate spores and theirs would seem to be the earliest name that can with confidence be applied to the species in question. *P. Briardi* Sacc. published in June 1892 may be the same fungus but is described as having smaller spores and as being associated with a different type of leaf spot. Salmon (1907) reported *P. Mali* on Peasgood Nonsuch, Cox's Orange Pippin and Ribston Pippin apple leaves in England. His figures show precisely the same type of spot as that dealt with here and also the typical, biguttulate, *Phyllosticta* spores. He made no mention of a *Coniothyrium*.

In the meantime the apple leaf-spot fungi may therefore be named:

- (1) *Phyllosticta Mali* Prill. & Del. 1890.

Syn. *P. tirolensis* Bub. 1904 (probably).

- (2) *Coniothyrium pirinum* (Sacc.) Sheldon, 1907.

Syn. *Phyllosticta pirina* Sacc. 1878.

Coniothyrium tirolense Bubák, 1904.

C. piricola Potebnia, 1907.

No great reliance can be placed on either name, however, as it seems most unlikely that either fungus is confined to leaves of apple and pear, or even to *Pyrus* leaves in general. Wollenweber and Hochapfel have already obtained *Coniothyrium tirolense* from dry twigs of *Juglans mandschurica* in the botanic garden, Berlin-Dahlem. It is quite likely that when adequate cultural studies of the Sphaeropsidales can be undertaken, each species will be found to have many other synonyms applied to fungi collected on other substrata, some of which may well antedate the names suggested above.

Crabill found both species to be non-parasitic to uninjured apple leaves; in his view they were saprophytes or facultative parasites on lesions initiated by *Sphaeropsis malorum*. It does not follow that the same condition obtains in Britain, as Crabill's fig. 18 of diseased apple leaves shows a 'frog-eye' type of spot with very broad brown border. This evidently represents a more serious disease than that represented by the Scottish material, on which there is no trace of a *Sphaeropsis*.

Thanks are due to the Director of the Imperial Mycological Institute for kindly providing the facilities which made continuation of this work possible after the junior writer's transference to Kew.

Strasseria carpophila Bres. & Sacc.

The occurrence of this fungus on black rotted apples in Edinburgh has already been reported elsewhere (Dennis, 1943).

Septocylindrium Aspidii Bresadola in *Hedwigia*, xxxv, 201 (1896).

Septocylindrium Aspidii forms a wide-spreading powdery white bloom on the upper surface of *Aspidium* fronds. The conidiophores are short, 6–8 μ long, 3 μ broad; the conidia cylindrical, straight or slightly curved, 15–24 \times 3 μ , multiguttulate, with one- to four-septa. The above diagnosis is based on that given by Bresadola; spores from a British collection on *Aspidium* sp., woods between Ceres and Cupar, Fife, 5 July 1944, measured 14–33 \times 2.5–3 μ but were not clearly septate. The disease was at that time widespread in East Fife and, as Bresadola observed, caused no well-defined spots but formed a conspicuous white bloom over extensive dead portions of the pinnae.

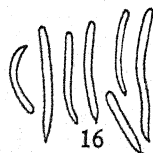


Fig. 16. *Septocylindrium Aspidii* Bres. Conidia ($\times c. 550$).

Dactylium dendroides Fr. subsp. *leptosporum* Sacc. in *Michelia*, II, 576, 1882.

The following description is based on growth in a malt agar culture of a fungus isolated by Dr W. A. Millard from diseased bracken fronds on a moor in Yorkshire, in September 1945.

Submerged hyphae hyaline, about 2–6 μ in diameter, rather sparingly septate; aerial hyphae forming a rather loose, short, buff-coloured turf. Conidiophores not clearly differentiated from the mycelium. 300 μ or more long, about 5 μ thick at the base, tapering to about 3 μ at the apex, sparingly septate, bearing up to four whorls of phialides, 3–6 (usually 3–4) in a whorl, individual phialides occasionally growing out to form branches with secondary whorls. Conidia dry, hyaline, smooth, oblong-elliptical, equally rounded at each end or slightly narrowed to the base, marked by a small apiculus, three-septate at maturity, 18–24 \times 5–6 μ .

These conidia appear to be somewhat narrower than those seen by Saccardo, whose diagnosis was: 'Conidiis paulo angustioribus, nempe 22 \times 8 μ oblongis, 3-septatis, basi apiculatis, hyalinis. In corticibus emor-

tuis udis Newfield, N. J.' In view of this discrepancy and the brevity of Saccardo's description, the identification of this fungus must be regarded with some reserve.

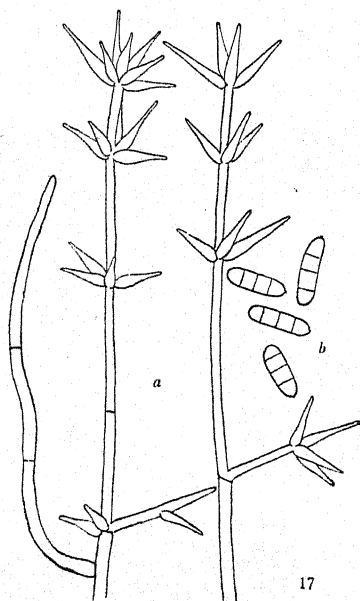


Fig. 17. *Dactylium dendroides* subsp. *leptosporum* Sacc., from malt agar culture one month old. a, terminal portion of two conidiophores; b, four detached conidia ($\times 400$).

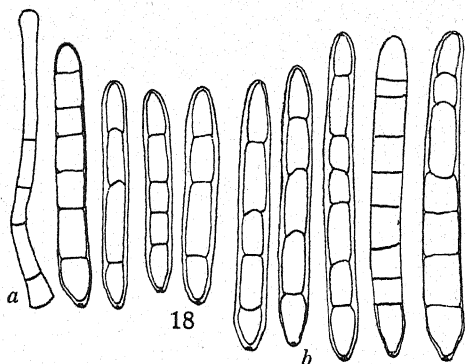


Fig. 18. *Helminthosporium tritici-repentis* Died. a, conidiophore; b, nine conidia from dead leaf of *Agropyron repens*, Kew ($\times 300$).

Helminthosporium tritici-repentis Diedicke ex Drechsler in *Journ. Agric. Res.* xxiv, 667 (1923).

The study of graminicolous species of *Helminthosporium* has been sadly neglected in Britain. There follow diagnoses of a number of species recorded, apparently for the first time in England, in the summer of 1945.

H. tritici-repentis has dark olivaceous conidiophores, $80-220 \times 7-9 \mu$, three- to six-septate, with a swollen basal segment; conidia subhyaline, straight cylindrical, one to nine times septate, $45-175 \times 12-21 \mu$ (Kew collection $98-160 \times 12-18 \mu$, two- to eight-septate). The basal segment of the spore tapers abruptly to the hilum, at which it becomes rounded off; the resulting profile recalls the head of a snake seen from above. The distal segment is usually rounded off in a hemispherical form but the two or three terminal segments may taper appreciably towards the distal end.

On dead basal leaves of *Agropyron repens*, without any distinctive lesions, Kew, 24 May 1945.

Helminthosporium dictyoides Drechsler in *Journ. Agric. Res.* xxiv, 679 (1923).

Conidiophores dark brown or olivaceous, $70-150 \times 6-8 \mu$, three- to six-septate, geniculate; conidia subhyaline, later yellow, typically straight

with maximum diameter at the basal segment, $14-17\mu$, tapering uniformly and very perceptibly to the apical segment, which in long spores often does not exceed $8-9\mu$ in diameter; less often approximately cylindrical or short ellipsoidal; length $23-115\mu$, usually $50-70\mu$, one- to seven-septate, typically three- to five-septate, not perceptibly constricted at the septa. Germination is typically by two germ tubes, one from each end segment, and produced usually at a right or oblique angle to the axis of the spore. Newly infected leaves show irregular brownish areas marked with dark longitudinal and transverse streaks, forming a delicate reticulate pattern.

The above diagnosis is condensed from that of Drechsler for a fungus on *Festuca elatior*. What appears to be the same species occurs at Kew on *Lolium* with conidia $60-150\mu$, mainly $75-105\mu$ long, basal cell $13-18\mu$ wide, apical cell $10-14\mu$ wide at the septum. It seems to be distinguishable from *Helminthosporium siccans* on the same host by the tapering spores, characteristic net blotch lesions and mode of germination, but the relationships of these two fungi obviously call for investigation.

Helminthosporium dematioideum Bubák & Wróblewski in *Hedwigia*, LVII, 337 (1916).

The brief original diagnosis of this species ran as follows: fructifications minute, scattered, pulverulent, black, mycelium dematioid, blackish brown. Conidiophores cylindrical, $25-60\mu$ long, $5-6\mu$ broad, subtorulose, septate, brown. Conidia cylindric-oblong, $39-42\mu$ long by $9-13\mu$ broad, three-septate at maturity, rounded at each end, yellow-brown, thick-walled, smooth, on glumes and pales of *Anthoxanthum odoratum*. Drechsler (1923) identified with this a fungus encountered in America on fading leaves of the same plant, having characteristically slender conidiophores and two- to six-septate spores $18-48\mu \times 8.5-14\mu$. What is clearly the same species was found on dead basal leaves of *A. odoratum* on Leith Hill, Dorking, 3 June 1945, with conidiophores $50-90 \times 6\mu$ and one- to four-septate spores $30-50 \times 9-16\mu$.

Helminthosporium triseptatum Drechsler in *Journ. Agric. Res.* xxiv, 686 (1923).

Fructifications scattered sparsely on withering leaves of *Holcus lanatus*, not associated with visible lesions. Sporophores arising singly or in pairs, $200-400 \times 6-8\mu$, usually six- to eleven-septate; proliferation of spores is associated with conspicuous local thickening of the peripheral wall, resembling a ring or band: these thickenings occurring in series give the upper portions of the sporophores a more or less moniliform contour. Spores dark olivaceous, ellipsoidal or short cylindrical with hemispherical ends, sometimes tapering somewhat towards basal end, regularly two- to three-septate, with an unusually thick peripheral wall, not contracted at the septa; $35-50 \times 15-21\mu$ (Surrey collection $34-45 \times 15-20\mu$). Germination is by production of one or two germ tubes at positions adjacent to the hilum.

The type host is *Holcus lanatus* and the fungus occurred on dead basal leaves of that grass at Hackhurst Downs, Surrey, 17 June 1945. It was

associated with another species having cylindrical to fusoid, four- to eleven-septate conidia $56-135 (-230) \times 10-16 \mu$, and bipolar germination. Of the graminicolous forms this seems nearest to *H. Leersii* but may well be an undescribed species. Drechsler (1935) ascribed a minor leaf spot of *Agrostis* spp. to *Helminthosporium triseptatum*.

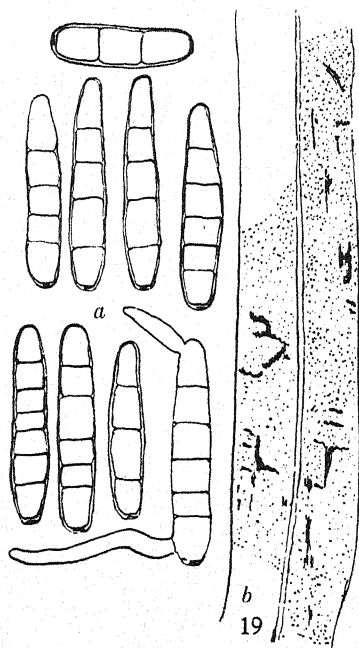


Fig. 19. *Helminthosporium dictyoides* Drechsler. a, nine conidia, one germinated after $4\frac{1}{2}$ hours in tap water ($\times 300$); b, net-blotch lesion on lower surface of *Lolium* leaf, yellow area stippled, necrotic areas in black. ($\times c. 4$).

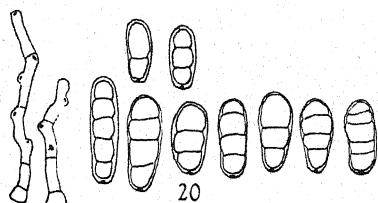


Fig. 20. *Helminthosporium dematioideum* Bub. & Wróbl. Conidiophores and conidia from faded leaf of *Anthoxanthum odoratum* ($\times 300$).

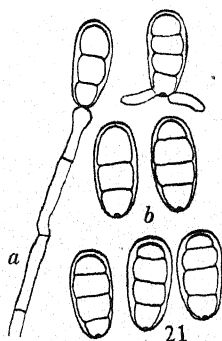


Fig. 21. *Helminthosporium triseptatum* Drechsler. a, conidiophore; b, six conidia, one germinated in tap water, from dead leaves of *Holcus lanatus* ($\times 300$).

Helminthosporium stenacrum Drechsler in *Journ. Agric. Res.* xxiv, 683 (1923).

Sporophores solitary or in pairs, geniculate, $80-250 \times 7-10 \mu$, three- to ten-septate. Spores subhyaline to yellowish, $53-135 \times 15-23 \mu$, subcylindrical with hemispherical or hemiellipsoidal ends, or widest somewhat below the middle and tapering moderately towards the ends; the apical portion sometimes produced into a somewhat narrowed distal prolongation, one- to eleven-septate, scarcely or not constricted at the septa. Germination is by production of germ tubes from several or all segments. To this species is tentatively ascribed a fungus collected on dead basal leaves of *Agrostis* sp., Kew Gardens, 15 June 1945. A number of species of *Helminthosporium* are known to occur on *Agrostis* but *Helminthosporium stenacrum* is characterized by and named for 'the somewhat attenuated distal prolongation characteristic of many of the spores', a character clearly displayed by several of

those figured here. As in Drechsler's experience, the conidiophores were found on dry withered leaves, not associated with any definite lesion. In this the Kew material seems to differ from *H. erythrospilum* described by Drechsler (1935) as causing a leaf spot of *Agrostis*.

Alternaria Anagallidis Raabe in *Hedwigia*, LXXVIII, 87 (1938).

Spots mainly along the edges of the leaves, rounded, brown, concentrically zoned, up to 1 cm. across; conidiophores sparse. Conidia pallid-

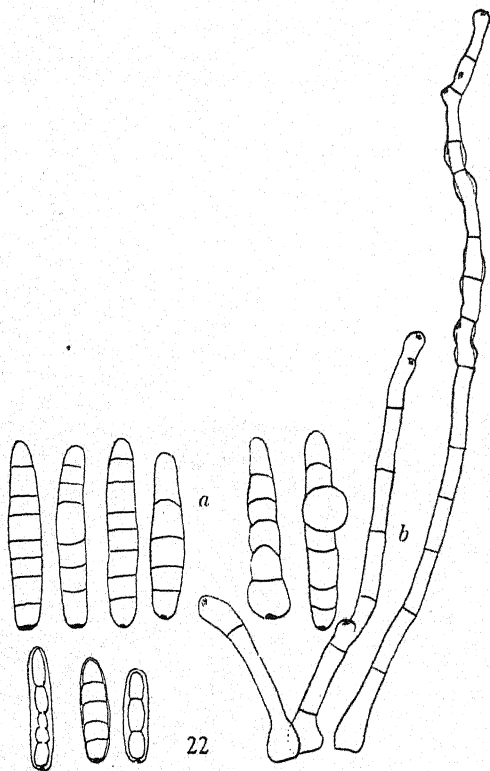


Fig. 22. *Helminthosporium stenacrum* Drechs. a, nine conidia from dead leaves of *Agrostis* sp.; b, three conidiophores ($\times 300$).

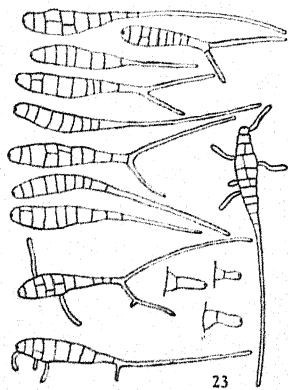


Fig. 23. *Alternaria Anagallidis* Raabe. Conidia from leaf-spots of *Anagallis arvensis* kept overnight in damp Petri dish; three germinated after 3 hours in tap water. Conidiophores from lesions collected out-of-doors ($\times 200$).

olivaceous, clavate, drawn out into a long beak which is sometimes forked, multiseptate, finally muriform, $95-235\mu$ long (including beak), $10-26\mu$ broad. On living leaves of *Anagallis arvensis* (scarlet pimpernel).

This fungus was very abundant in July 1945 on living leaves of scarlet pimpernel at Kew. In addition to the foliar lesions mentioned by Raabe the stems were often attacked and girdled, resulting in the death of whole branches. Conidia, rather sparsely produced on the lesions out of doors, appeared in profusion on those kept overnight on moist filter paper in a Petri dish. The conidia in the Kew material measured $100-180 \times 12-15\mu$.

Neergaard (1945), who obtained *Alternaria Anagallidis* on *Anagallis*

arvensis and *A. arvensis* var. *caerulea* at Copenhagen and studied it in agar culture, remarks that it resembles the forms of *Alternaria Porri* (his name for the group of allied species including *A. Solani*), but differs in the considerably shorter spore-body, in cultural characters and in host relationships. In his infection experiments the fungus attacked seedlings of *Linaria maroccana* and, to a slight extent, lettuce, onion, *Dianthus*, *Godetia*, *Nicotiana* and *Antirrhinum*.

Oidiodendron fuscum Robak in *Nyt Mag. for Naturvidenskab.* LXXI, 249 (1932).

In July 1943 one of us (R. W. G. D.) collected from a prostrate log on Corstorphine Hill, Edinburgh, a patch of bark several inches long bearing a copious growth of a short grey mould which proved of much interest. In general appearance it recalled *Hormodendron cladosporioides*, now regarded as a phase of *Cladosporium herbarum*. Spores planted on 2% malt agar, however, yielded cultures quite unlike *Cladosporium* and consisting of a slow-growing grey-brown mat with a distinct tendency to become lumpy and heaped up at intervals. The under-surface of the cultures appeared deep brown and finally almost blackish brown though never quite black, due to a change in colour of the medium rather than to that of the hyphal mat. Microscopic examination revealed abundant tall brown conidiophores, much branched at the top and ultimately crowned by densely clustered chains of unicellular grey-brown conidia. The fungus is evidently a species of *Oidiodendron*, established by Robak in 1932. He described it as resembling *Hormodendron*, from which it differed in the conidiophore branches dividing into equal segments which rounded off as spores. It was said to be 'nearest to a further development of the type which is represented by the genus *Oidium* (Link) Sacc. and by species of the genus *Torula* Pers. where the spores develop in chains by the division of the ordinary hyphae'. This type of spore formation is clearly shown by cultures of the Scottish material. Robak recognized three species, *Oidiodendron nigrum*, *O. fuscum* and *O. rhodogenum*. The latter cannot be the species from Scotland as it has hyaline mycelium and usually forms a red stain in the medium or on wood pulp. Of the others, the Corstorphine fungus agrees better with *O. fuscum* which is distinguished from *O. nigrum* by its smaller spores ($1.6-3.5 \times 1.2-2.2 \mu$ instead of $2.2-5.4 \times 2-3.7 \mu$), longer conidiophores, more clearly differentiated from the mycelium, and by the colour of the conidial layer on wood pulp, which is grey-brown to chocolate brown instead of sooty black. The diagnosis is as follows:

Oidiodendron fuscum Robak. Immersed hyphae $1-1.5 \mu$ thick, aerial hyphae usually slightly coarser, smooth, at first pale brown, later dark brown. Conidia brownish green or dark blue-grey, occasionally hyaline, $1.6-3.6 \times 1.2-2.2 \mu$, average $2.4 \times 1.7 \mu$. Conidiophores tree-like, glabrous 60-200, seldom up to 265μ and mostly 100-110 μ long, main stem cylindrical, $1.5-2 \mu$ thick, unbranched for more than half its length. At the edge of the cultures single chains of slightly larger conidia may occur.

Fusarium Poae (Peck) Wollenw. apud Lewis, *Maine Agric. Expt. Stat. Bul.* 219 (1913).

Sporotrichum Poae Peck in 56th *Ann. Rept N.Y. State Museum*, 29 (1904) (for full synonymy see Wollenweber and Reinking, 1935).

A fungus isolated from pink-stained oats from an Aberdeenshire crop was determined as *Fusarium Poae* by Dr G. R. Bisby, who was familiar with the species in Canada. The same organism had been collected previously on a cock's-foot (*Dactylis glomerata*) head much damaged by insects at Corstorphine. In cultural characters it agrees with the description given by Wollenweber and Reinking: Growth cobwebby or a woolly felt, white or rosy, consisting of much-branched hyphae and conidiophores, branches alternate, dichotomous or whorled, stroma colours carmine-red-purple, ochre-yellow or violet. Conidia forming an isabelline or whitish powder over the mycelium or clustered into a sandy or mealy layer on the substrate.

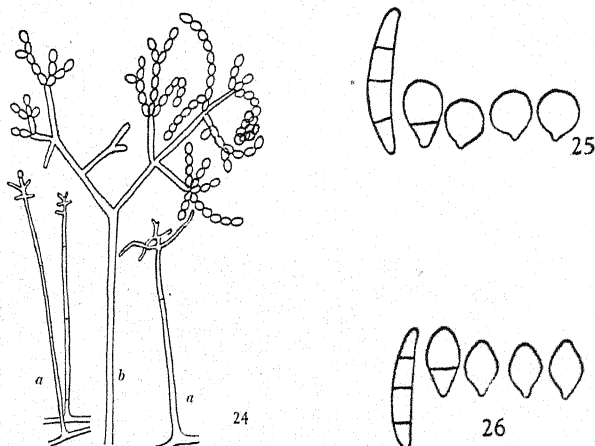


Fig. 24. *Oidiendendron fuscum* Robak, from 18 days' old culture on malt agar. *a*, three conidiophores mounted in lactophenol, spores removed, showing branching ($\times 400$); *b*, apical portion of conidiophore with spores attached ($\times 800$).

Fig. 25. Conidia of *Fusarium Poae* (Peck) Wollenw. apud Lewis ($\times 750$).

Fig. 26. Conidia of *F. citriforme* Jamalainen. ($\times 750$, reduced from Jamalainen, 1943.)

In the original description of the species by Peck, only subglobose, hyaline conidia $4-8\mu$ in diameter were mentioned, hence it was classed as a species of *Sporotrichum*. Similarly only *Sporotrichum*-like conidia have been seen either on the cock's-foot head or in the cultures derived from it and from the discoloured oats. Wollenweber and Reinking, however, obtained in their cultures up to 4% of conidia with one- to four-septa and consequently transferred the species to *Fusarium*. Their conidia were nearly spherical, lemon-shaped (the form with lemon-shaped conidia has been separated by Jamalainen as *F. citriforme*), or pear-shaped, non-septate, $5-12 \times 3-8\mu$ or one-septate and $9-20 \times 3.5-9\mu$, occasionally longer, spindle-shaped, non-septate $10-15 \times 2.5-4\mu$ or one-septate $12-26 \times 2.7-5\mu$ or sickle-shaped and three-septate, $18-35 \times 3.5-5\mu$.

Peck's original collection was on sheaths and culms of *Poa pratensis*, but

Sprague has reported this species as discolouring oat panicles in Oregon and one of Wollenweber's collections was on oat. The fungus is also regarded as the cause of bud rot of carnation and has been found associated with blighted ears of *Agrostis*, *Avena byzantina*, *Bouteloua gracilis*, *Bromus inermis*, *Phleum*, *Poa*, barley and wheat in North America and with foot-rot of rye in Italy. Other records are as a saprophyte on peas injured by foot-rot and root-rot in U.S.A., as an associate of root-rots of lucerne, *Medicago falcata* and *Melilotus* spp. in Canada, on red clover seed in U.S.S.R., on stored citrus fruit in Georgia and on ripe peaches in Italy. Its known distribution includes U.S.A., Canada, Argentina, New South Wales, U.S.S.R., Italy and Germany and Jamalainen (1943) has recently reported it from Britain. Oat seed inoculated with the culture from Aberdeenshire oats gave a full stand of healthy seedlings, so the species seems unlikely to be of economic importance as a parasite of British cereals. It should be noted, however, that most of the foreign records indicate that the part attacked in the Gramineae is the maturing panicle, usually in association with the grass mite *Pediculopsis graminum*.

Jamalainen (1943) also recorded his closely related species *Fusarium citrifforme* Jamalainen 'In cariops Tritici, Britannia, Newcastle-upon-Tyne, 1935, leg. Bennet'. This species differs from *F. Poae* in its lemon-shaped one-celled conidia.

Note. Since the description of *Psilocybe cyanescens* was sent to press, our attention has been called to a figure of *Hypholoma cyanescens* R. Maire (1928) in *Bull. Soc. Mycol. Fr.* LVIII, 1942, with a redescription of the fungus by Malencon on p. 49. This North African fungus resembles ours very closely, and may prove to be the same. It differs however in certain points, notably in the more campanulate pileus (according to Malencon) and in the adnexed gills. It is noteworthy that Malencon suggests relationship to certain species of *Psilocybe*.

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UROMYCES STRIATUS SCHROET. ON *MEDICAGO LUPULINA* L. AND OTHER HOST PLANTS IN BRITAIN

BY H. H. GLASSCOCK AND W. M. WARE

A seven acres field of trefoil (*Medicago lupulina* L.) near Faversham, Kent, was visited by us on 17 October 1945. As far as could be ascertained, every plant was infected by a rust which on microscopic examination was found to agree with a description (Grove, 1913) of the uredo and teleuto stages of *Uromyces striatus* Schroet. The attack was particularly intense in large, well-defined patches where the leaves of the plants had been killed and where mature pustules were so abundant that when the plants were disturbed by walking amongst them, a cloud of spores rose in the air and a visible deposit was left on boots and clothing. These areas of very heavy infection corresponded to places where the previous wheat crop had been thinner and where the ley had 'taken' first with more numerous plants. Pustules of the fungus were present on both sides of the laminae but were far more abundant on the lower surface. They were also seen on petioles, stipules and peduncles.

No sign of the fungus could be found on an adjoining crop of lucerne (*Medicago sativa* L.), a plant which is commonly attacked by *Uromyces striatus* in the U.S.A. and in parts of Europe.

The farmer reported that a second field of twenty acres of trefoil, situated a few miles away, and grown from seed of the same delivery, was similarly infected. Damage to both crops was so severe that they were useless for forage and would, therefore, be ploughed in.

The aecidial stage of *U. striatus* is reported by Grove (1913) to occur on *Euphorbia Cyparissias* L. but to be unknown in Britain. This alternate host which is stated by Butcher (1930) to be 'a not uncommon casual weed, especially in E. England and possibly a native', was not looked for at the time of our visit.

A second outbreak of *Uromyces striatus* on trefoil, this time in the West of England, was observed by Mr L. Ogilvie and Dr Moira C. D. Munro who inform us that they found it in experimental plots at Chedzoy near Bridgwater, Somerset, on 29 November 1945.

A search was made of the literature for previous mention of *U. striatus* on trefoil and on other hosts in Britain but only two (of doubtful value) relating to trefoil were found. One of these is in the *List of Common British Plant Diseases* (all editions) compiled by the Plant Pathology Committee of the British Mycological Society; the other is that by Ainsworth (1937) who cites Grove (1913). The sole statement made by Grove (1913) which can refer to trefoil is that '*U. striatus* is found... on many species of *Medicago*, including all the British species, but I have seen no specimens on these from this country'.

Actual records of *Uromyces striatus* on other hosts (Grove, 1913) are 'uredo- and teleutospores on leaves and stems of *Trifolium minus*, Bath (Herb. Broome); King's Norton (Worcestershire). Very uncommon. July-August.... The teleutospores on *T. minus* which I have observed are more distinctly verrucose and less striated than in the figures given by Fischer, and may possibly not belong to the same species.' Ramsbottom (1914) pointed out: 'The species of *Uromyces* on *Trifolium minus* in the Broome Herbarium has been found on examination to be *U. Jaapianus* Kleb. This was previously lumped with *U. striatus*. The other record in Grove is also on *T. minus*, and is also probably *U. Jaapianus*.' It should be noted here that three of the spores figured by Grove as *U. striatus* (ex Herb. Broome) are now known to be spores of *U. Jaapianus*.

Another mention of *U. striatus* on *Trifolium minus* is made in a list, compiled by Ramsbottom (1921), of Uredineae found at Porlock during the Minehead foray of the British Mycological Society, 27 September-2 October 1920.

In connexion with this foray, Dr Alex Smith of the Plant Pathology Laboratory, Harpenden, informs us *in litt.* that a specimen exists at that laboratory originally labelled *Puccinia striatus* on *Medicago arabica*. This specimen was collected at the Minehead foray in 1920. In the absence of teleutospores (which are always uncommon on this host) identification is less certain, but the name on the label has been changed to *Uromyces striatus* because the original was presumably a *lapsus calami*. Dr Smith further informs us that because no mention is made of *Medicago arabica* as a host plant in the published list of fungi collected on the foray (Ramsbottom, 1921), he sent the specimen to the Royal Botanic Gardens, Kew, where its identity as *M. arabica* was confirmed.

Thus, resulting from the Minehead foray, there is a hitherto unrecorded specimen of *Uromyces striatus* on *Medicago arabica* in the Herbarium at Harpenden and a record (Ramsbottom, 1921) of *Uromyces striatus* on *Trifolium minus* but with no statement as to the existence of any herbarium specimen.

Uromyces striatus on *Trifolium minus* is further listed by Hadden (1920) as occurring on a lawn at Porlock. It is not known whether this record is distinct from that by Ramsbottom (1921) already cited from Porlock. We have received from Mr Hadden a specimen of the fungus which he listed in 1920. Uredospores only were present; they showed that the fungus was *Uromyces Jaapianus*. The specimen was sent to the British Museum (Natural History) and our opinion was confirmed by Dr Ramsbottom.

In a table showing characteristics of the Rusts of British herbage legumes, Sampson and Western (1941) included *U. striatus* on *Medicago maculata*. We are informed by one of these authors that this host plant was cited by them as a result of a communication from Dr Alex Smith who was referring to the specimen of *M. arabica* collected at the Minehead foray. The synonym *M. maculata* was used.

Hadden (1916) made what he considered to be the first record in Britain of *Uromyces striatus* on *Trifolium procumbens* from specimens found at Hollerday Hill and Lynbridge Lane, north Devon. A specimen of this was re-

ceived from Mr Hadden. Teleutospores were absent but the uredospores showed that the fungus was *Uromyces Jaapianus*. This identification was also confirmed at the British Museum (Natural History).

Thus, in the literature to which reference has been made, it appears that existing records of *U. striatus* stand for only one host in Britain, namely *Medicago arabica*. From the fact that every British specimen of *Uromyces striatus* on *Trifolium* spp. examined by us has proved to be *Uromyces Jaapianus*, it would seem that all published records of *U. striatus* occurring on *Trifolium* spp. will need confirmation.

Klebahn (1913), under his new species *Uromyces Jaapianus*, included (pp. 239-40) only the one specimen on *Trifolium minus* collected by Jaap. On pp. 244-5 under *Uromyces striatus* he still included as hosts *Trifolium agrarium*, *T. minus*, *T. procumbens* and *T. arvense*, but it is not known whether he had examined critically the exsiccati he enumerated.

Uromyces striatus is now recorded for the first time on a crop plant, *Medicago lupulina*, in Britain. Specimens have been sent to the Herbaria at Kew and the British Museum.

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PREDACIOUS FUNGI IN BRITAIN

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In 1940, I recorded the occurrence of four species of Zoopagaceae in this country, namely, *Cochlonema verrucosum* Drechsl., *C. dolichosporum* Drechsl., *Stylopaga hadra* Drechsl., and *S. haploe* Drechsl. All four species were obtained from samples of leaf-mould from Guiting Wood, near Winchcombe, Gloucestershire.

Since that time, further observations have been made, and these have yielded the following first records for this country:

(1) *Cystopaga lateralis* Drechsl., a member of the Zoopagaceae, capturing and consuming nematodes in a sample from an old manure heap at Coppice Corner Farm, Lowsonford, Warwickshire.

(2) *Protascus subuliformis* Zopf, attacking nematodes in the same sample which yielded *Cystopaga lateralis*.

(3) *Stylopaga* species, capturing and consuming small amoebae. The mycelium consisted of delicate hyphae, $0.7-1.2\mu$ in diameter, bearing ovoid conidia on slender conidiophores which, by continued growth, might bear more than one conidium. The amoebae were held on the mycelium, apparently by some sticky substance, and their contents were absorbed by the fine, branched haustoria. The species could not be determined with certainty because of the lack of mature material, but it seemed to be near to *S. lepte* Drechsl. It occurred on rotting plant debris from the margin of a pond at Bushwood Farm, Lowsonford, Warwickshire (Map reference, O.S. 1/63360, sheet 72, 637890).

(4) *Meria coniospora* Drechsl., attacking nematodes in a sample of well-rotted cow dung from a manure heap at Coppice Corner Farm, Lowsonford.

(5) *Harposporium anguillulae* Lohde., parasitic on nematodes, has been observed twice; in leaf mould from Brick Kiln Hole Wood, Solihull, Warwickshire, and in old cow dung from Coppice Corner Farm, Lowsonford.

(6) *Dactylella bembicoides* Drechsl., capturing nematodes by means of constricting rings, in old cow dung from Coppice Corner Farm, Lowsonford.

Work is in progress on these and other forms, and it is proposed at a later date to give further details of the systematics and biology of British predacious fungi.

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A MULTIPLE-POINT INOCULATING NEEDLE FOR AGAR PLATES

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(With 1 Text-figure)

Recent work in this laboratory has required the inoculation of agar plates at ten points with agar mycelial disks of a non-sporing fungus. As at least a hundred plates had to be inoculated at one time, ten separate transfers of agar disks to each inoculant plate would have entailed not only waste of time, but also considerable risk of contamination, in opening and closing the Petri dishes so many times. A ten-point inoculating needle was therefore devised and constructed, as follows. Ten 15 cm. lengths of steel

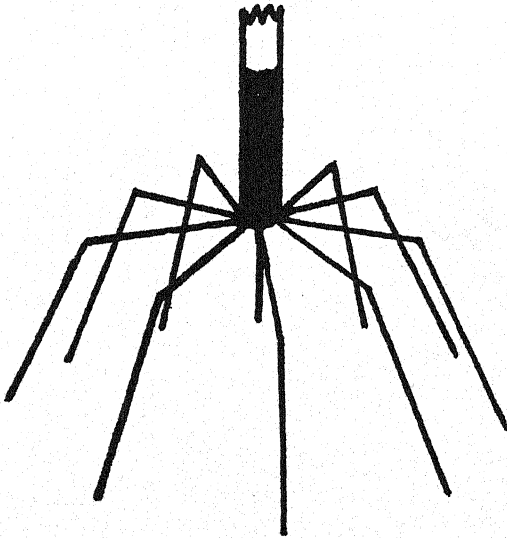


Fig. 1

wire, No. 20 gauge, were cut, and one end of each was hammered out flat on an anvil. Each length of wire was bent through an angle of 60° at a distance of 6 cm. from the flattened end, and then through an angle of 90° in the reverse direction at a distance of 10 cm. from the same end, both angles being in the plane of the flattened tip of the wire. The ten lengths of bent wire were then clamped to a wooden holder, in which grooves had been cut, to give the finished instrument (see Fig. 1).

After sterilizing the ten points in a flame, the instrument was used as follows. The inoculum plate, in which agar disks had been cut with a

sterile cork borer, was held upside down without its lid above eye level, and an agar disk removed on the spatulate end of one of the ten 'points'. The instrument was given a slight twist and a second disk was removed on the next point, and so on until all ten points were carrying disks. The inoculum plate was then replaced in its lid, and the inoculant plate was picked up and held in a similar position, while the ten inoculum disks were transferred one by one to their correct positions, previously marked out by means of a stencil on the bottom of the dish.

The number of plates thus inoculated which have to be discarded owing to contaminations does not much exceed that lost when plates are inoculated at a single central point. In the first trial of this technique, the inoculation of sixty-five plates took four hours, and six plates each developed a single contaminant colony, but of these only two plates had to be discarded as useless.

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A SUBSTANCE CAUSING ABNORMAL DEVELOPMENT OF FUNGAL HYPHAE PRODUCED BY *PENICILLIUM JANCZEWSKII* ZAL.

I. BIOLOGICAL ASSAY, PRODUCTION AND ISOLATION OF 'CURLING FACTOR'

By P. W. BRIAN, P. J. CURTIS AND H. G. HEMMING

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(With 2 Text-figures)

In the course of an investigation of the toxicity of Wareham Heath soil (Brian, Hemming & McGowan, 1945) it was noted that the mould flora was composed almost entirely of species of *Penicillium*, as had been previously recorded by Neilson-Jones (1941). It was further shown that these species, with but a few exceptions, fall into three species or groups:

(a) *P. Janczewskii* Zal.

(b) *P. Jensenii* Zal.

(c) A group of strains falling into the *P. nigricans-Janczewskii* series of Thom (1930) but distinct from those identified as *P. Janczewskii*.

P. Jensenii was shown to produce the antibiotic substance known as gliotoxin. The strains in group (c) produced an antibiotic which had not at that time been isolated and the strains of *P. Janczewskii* produced in liquid culture-media a substance having a peculiar distorting effect on the germ tubes of *Botrytis Allii*. The present communication is concerned with more detailed investigations of the production and properties of this latter substance.

CULTURAL CHARACTERISTICS OF *PENICILLIUM JANCZEWSKII*

Colonies on Czapek-Dox agar develop a closely meshed felt almost velvety in appearance. The colonies show radial folds and a convoluted and raised centre, with a white margin about 1 mm. wide. Conidial areas are greenish-grey, becoming almost black and the reverse yellow, ochraceous brown, or purplish brown. The odour is strong and reminiscent of *P. expansum*. The penicillus is of the divaricate type characteristic of the *Lanata-Divaricata*, showing a main axis with divergent branches. The sterigmata are 7 μ long, the conidia spherical and roughened, 2.5-3 μ in diameter. Four strains could be distinguished, varying slightly in the colour of the colony reverse, but all the work described below has been carried out on one strain only (no. 250). These strains were kindly examined by Dr K. B. Raper (United States Dept. of Agriculture) who agrees that they fall into the *nigricans-Janczewskii* series, but makes the point that it is questionable if one can differentiate satisfactorily between the species *nigricans* and the species *Janczewskii*.

NUTRIENT REQUIREMENTS OF *PENICILLIUM JANCZEWSKII*

P. Janczewskii grows well on the ordinary synthetic media. It can utilize either ammonium salts or nitrates as a nitrogen source; growth is more rapid with an ammonium nitrogen source. A wide variety of substances can be utilized as a source of carbon. Only a limited range of sugars have been tested, but growth is good, in a medium of the Czapek-Dox type, with dextrose, sucrose, maltose, lactose, galactose, dextrin and starch. Growth is also good where glycerol or succinic acid are used as carbon sources. Less suitable, but utilizable by the mould, are fumaric acid, malic acid, lactic acid, acetic acid, citric acid, aconitic acid and malonic acid. The mould is not able to utilize racemic acid; mesotartaric acid, tartaric acid, glycollic acid, meconic acid, maleic acid, diethylene glycol, oxalic acid or itaconic acid. The failure to utilize tartaric acid is unusual.

PRELIMINARY OBSERVATIONS ON ANTIBIOTIC ACTIVITY

(1) *Antagonism in culture.* Plates of Lemco-peptone-glucose agar (Lab-Lemco 3 g., peptone (Evans') 10 g., glucose 10 g., sodium chloride 5 g., agar 20 g., water 1000 ml.) were inoculated with a transverse streak of spores of *P. Janczewskii* and incubated for four days at 25° C. when a vigorous colony had been produced. Cell suspensions of three bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*) and one pathogenic fungus (*Endomycopsis albicans*) were then streaked at right angles to the mould colony, starting at its edge. The plates were then incubated for a further two days at 37° C. No inhibition of the growth of the test organisms was observed. With moulds producing antibiotics (e.g. *P. Jensenii* mentioned above) a marked zone of inhibition near the mould colony is usually seen.

(2) *Cultures on liquid media.* The following five media have been used:

Weindling (dextrose 25.0 g., ammonium tartrate 2.0 g., potassium dihydrogen phosphate 1.0 g., magnesium sulphate, hydrated 1.0 g., minor element concentrate 1 ml., water 1000 ml.; adjusted to pH 3.5 with hydrochloric acid).

Czapek-Dox (dextrose 50.0 g., sodium nitrate 2.0 g., potassium dihydrogen phosphate 1.0 g., potassium chloride 0.5 g., magnesium sulphate, hydrated 0.5 g., ferrous sulphate 0.01 g., water 1000 ml.).

Raulin-Thom (dextrose 50.0 g., tartaric acid 2.66 g., ammonium tartrate 2.66 g., diammonium hydrogen phosphate 0.4 g., potassium carbonate 0.4 g., magnesium carbonate 0.27 g., ammonium sulphate 0.17 g., zinc sulphate, hydrated 0.05 g., ferrous sulphate 0.05 g., water 1000 ml.).

Cornsteep (crude glucose 20.0 g., lactose 20.0 g., sodium nitrate 3.0 g., potassium dihydrogen phosphate 0.5 g., magnesium sulphate, hydrated 0.25 g., ferrous sulphate, hydrated 0.01 g., zinc sulphate, hydrated 0.002 g., cornsteep liquor 80 ml., water 920 ml.).

Lemco-dextrose broth (peptone (Evans') 10 g., Lab-Lemco 3 g., dextrose 10 g., sodium chloride 5 g., water 100 ml.).

The minor element concentrate has the following composition: ferrous

Abnormal Development of Fungal Hyphae. Brian and others 175

sulphate (hydrated), 0.1 g.; copper sulphate (hydrated), 0.015 g.; zinc sulphate (hydrated), 0.1 g.; manganese sulphate (hydrated), 0.01 g.; potassium molybdate, 0.01 g.; distilled water, 100 ml.

The media were disposed in 30 ml. lots in 100 ml. Pyrex flasks, autoclaved, inoculated and incubated at 25° C. Five flasks of each medium were withdrawn at random and bulked after six, eight, ten and twelve days' growth and the filtered liquid medium assayed for antibiotic activity. The assays used were serial dilution tests with two bacteria (*Staphylococcus aureus* and *Salmonella typhi*), carried out in the conventional manner, and a serial dilution spore germination test using conidia of *Botrytis Allii* Munn. The technique of this spore germination assay has been previously described by Brian and Hemming (1945). Results of an experiment with *Penicillium Janczewskii* (no. 250) are shown in Table 1. The data given show the greatest dilutions inhibiting germination of *Botrytis Allii* conidia (i.e. B.A. units/ml.) or growth of *Staphylococcus aureus* (i.e. *Staph.* units/ml.) or *Salmonella typhi* (i.e. *Salmonella* units/ml.). Experiments with other strains of *Penicillium Janczewskii* gave similar results.

Table 1. *Fungistatic and antibacterial activity of culture filtrates from Penicillium Janczewskii grown at 25° C. on five media*

Activity	Days' growth	Weindling	Raulin-Thom	Czapek-Dox	Cornsteep	Lemco-dextrose
B.A. units/ml.	6	4	8	2	—	—
	8	4	4	4	—	—
	10	4	—	2	—	—
	12	—	—	—	—	—
	12	—	—	—	—	—
<i>Staph.</i> units/ml.	6	4	4	4	—	—
	8	2	2	2	—	—
	10	2	2	2	—	—
	12	4	2	4	—	—
	12	4	2	2	—	—
<i>Salmonella</i> units/ml.	6	2	2	2	—	—
	8	2	2	2	—	—
	10	2	2	2	—	—
	12	2	2	—	—	—
	12	2	2	—	—	—

The antibiotic activity of the culture filtrates was relatively small throughout. The antibacterial effects of culture filtrates from Weindling, Raulin-Thom and Czapek-Dox media may probably be attributed to toxic effects of the media themselves, including the effects of acidity. The antifungal activity, though slight, is probably real. The relatively low level of antifungal activity is better appreciated when it is recalled that culture filtrates from appropriate strains of *Trichoderma viride* may reach an activity of 16,384 B.A. units/ml. (Brian, Curtis, Hemming & McGowan, 1946). In spite of the low level of fungistatic activity, as expressed in prevention of germination of conidia of *Botrytis Allii*, the culture filtrates had a marked effect on the growth form of *B. Allii*, on some media even in dilutions of 1 in 512. This took the form of stunting, distortion and increased branching in the lower dilutions of culture filtrate and a 'waving' of the hyphae in the higher dilutions. This was most marked, on this occasion, in culture filtrates from Cornsteep and Lemco-dextrose media, slightly less marked in culture filtrates from Czapek-Dox but only visible in the lower dilutions (1 in 8 and 1 in 4) of Weindling and Raulin-Thom medium.

Penicillium Janczewskii was shown by this experiment to produce a substance, conveniently designated 'curling factor', causing abnormal development of the young hyphae of *Botrytis Allii* in low concentrations. This remarkable effect, unique in our experience of culture filtrates from over two hundred moulds, appeared to be worthy of further investigation, aimed particularly at isolation of 'curling factor' in pure form. This necessitated the development of some form of quantitative assay; the procedure adopted is described in the following section.

BIO-ASSAY OF 'CURLING FACTOR'

Fig. 1 shows the appearance of *Botrytis Allii* conidia after incubation for seventeen hours at 25° C. in serial dilutions of a *Penicillium Janczewskii* (No. 250) culture filtrate after 14 days' growth on Czapek-Dox medium (10% glucose supplemented by 0.1% peptone). The dilutions were made in half-strength Weindling solution in the manner described by Brian & Hemming (1945). This example may be regarded as typical.

It will be seen that the effect of the 'curling factor' produced by *Penicillium Janczewskii* is to cause (a) stunting of the germ-tubes, (b) distortion and excessive branching of the hyphae, (c) waving of the hyphae produced. Stunting, distortion and excessive branching of the hyphae are seen most noticeably in the first dilutions of the culture filtrate, and waving of the hyphae in the greatest dilutions. It must be emphasized that, in these concentrations of 'curling factor', percentage germination is not affected. Even where there is most marked stunting (Fig. 1 A) germ-tubes were first produced and germination reached 100% at the same time as the controls (Fig. 1 I), that is in about seven hours. The effect of 'curling factor' in such concentrations is to prevent further development after the germ tubes have been formed. It is typical of the stunted hyphae (Fig. 1 A-F) that the diameter of the hyphae is much greater than normal and that the ends of the hyphae are frequently swollen or spatulate. The distortion and excessive branching, typical examples of which are seen in Fig. 1 E and F, are associated with stunting. It appears that hyphal branching is not inhibited, or is even stimulated, but that hyphal elongation is greatly retarded. In addition, the hyphae appear to lose their sense of direction and, instead of progressing away from the point of origin, tend frequently to turn back again. The waving seen in the greater dilutions (Fig. 1 G and H) is quite characteristic, but as the culture filtrate is diluted still further growth passes imperceptibly to the normal (Fig. 1 I).

After consideration of the gradual change in the effects on spore germination produced by 'curling factor' as dilution proceeds, it will be appreciated that the development of a satisfactory quantitative assay is not easy. In the case of a spore-germination test, it is relatively easy to make a distinction between germinated spores and those that have not germinated. Here the procedure adopted has been to determine (a) the greatest dilution at which obvious stunting has been caused (e.g. in Fig. 1 F but not G) and (b) the greatest dilution at which waving of the hyphae is produced. Both of these points have to be assessed arbitrarily and the result is necessarily over-dependent on subjective influences. Nevertheless it has been possible,

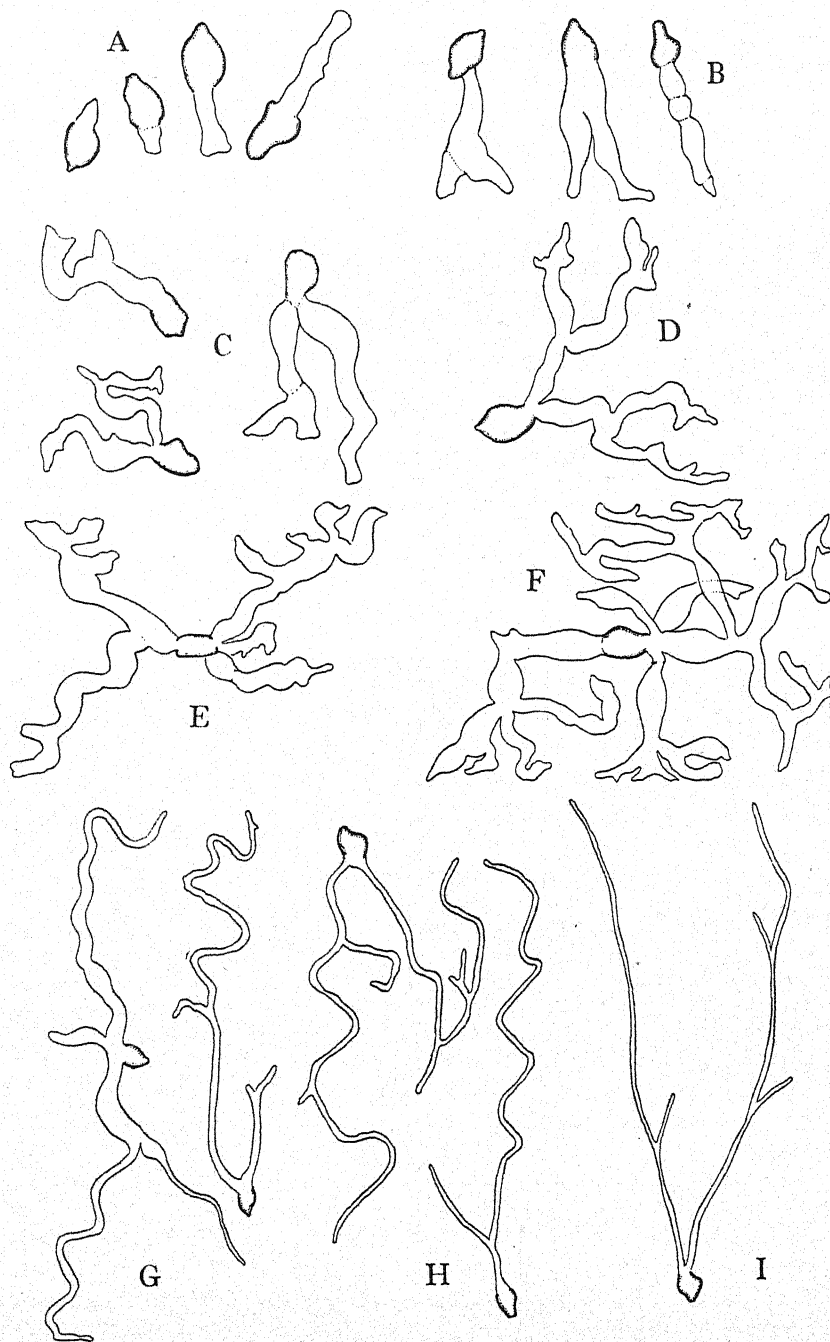


Fig. 1. Effect of various dilutions of a *Penicillium janczewskii* culture filtrate (Czapek-Dox + 0.1% peptone) on germination of *Botrytis Allii*. Drawings made after seventeen hours' germination at 25° C. Dilutions: A, 1 in 4; B, 1 in 8; C, 1 in 16; D, 1 in 32; E, 1 in 64; F, 1 in 128; G, 1 in 512; H, 1 in 2048; I, control. (A-F $\times 1.5$; G $\times 1$.)

on the basis of observations of this kind, to follow the production of 'curling factor' and to develop means of extraction and purification. Results are presented as in Table 2, where, for instance, $S_{32}C_{1024}$ indicates that stunting was clearly observed in dilutions down to 1 in 32, and waving of hyphae down to a dilution of 1 in 1024.

RELATION OF MEDIUM COMPOSITION TO PRODUCTION OF
'CURLING FACTOR'

Nitrogen source

As has already been stated (see discussion of Table 1), Czapek-Dox appeared to be a more favourable medium for production of 'curling factor' than Raulin-Thom in preliminary experiments, and the cornsteep medium better than Czapek-Dox. Further experiments indicated that cornsteep was not significantly superior to Czapek-Dox but the superiority of Czapek-Dox over Raulin-Thom was confirmed. This superiority is well demonstrated by the data presented in Table 4. The main difference between these two synthetic media lies in the nitrogen source, being in the form of sodium nitrate in Czapek-Dox and various ammonium salts in Raulin-Thom. The relation between nitrogen source and 'curling factor' production was therefore studied in greater detail.

The media used were somewhat similar to Czapek-Dox in composition, and nitrogen was supplied as potassium nitrate, ammonium nitrate, ammonium tartrate or Bacto-tryptone in amounts giving equal nitrogen concentrations. The detailed compositions of the media used are given below:

Medium N:

Potassium nitrate	2.3 g.
Potassium dihydrogen phosphate	1.0 g.
Magnesium sulphate, hydrated	0.5 g.
Dextrose	75.0 g.
Minor element concentrate	1 ml.
Distilled water	1000 ml.

Medium AN: as *N* but with potassium nitrate replaced by 0.95 g. ammonium nitrate.

Medium A: as *N* but with potassium nitrate replaced by 2.2 g. ammonium tartrate.

Medium T: as *N* but with potassium nitrate replaced by 2.5 g. Bacto-tryptone.

Medium NP: as *N* but with potassium dihydrogen phosphate increased to 10.0 g./l.

Medium ANP: as *AN* but with potassium dihydrogen phosphate increased to 10.0 g./l.

Medium AP: as *A* but with potassium dihydrogen phosphate increased to 10.0 g./l.

Medium TP: as *T* but with potassium dihydrogen phosphate increased to 10.0 g./l.

Abnormal Development of Fungal Hyphae. Brian and others 179

These media were all adjusted (when necessary, viz. *N*, *AN*, *A*, *T*, *AP* and *TP*) to pH 4.4 with *N*/1 hydrochloric acid before autoclaving; 500 ml. lots of each were disposed in 'Glaxo' culture vessels with six replicates of each, inoculated with *Penicillium Janczewskii* spores and incubated at 25° C. Periodically samples were taken from each culture vessel, samples from similar media bulked, assayed for 'curling factor' activity and the pH determined electrometrically. Results are shown in Table 2.

There were minor growth differences on these media. After three days' growth all were pure white, with no visible sporulation, a completely confluent mycelial felt having been formed on all media except *N* and *NP*, where the growth still consisted of numerous mycelial 'islands' which had not yet fused. By visual observation the order of growth vigour was

Table 2. Development of 'curling factor' activity in cultures of *Penicillium Janczewskii* on media with various sources of nitrogen

Days' growth	Medium							
	<i>N</i>	<i>NP</i>	<i>A</i>	<i>AP</i>	<i>AN</i>	<i>ANP</i>	<i>T</i>	<i>TP</i>
	Activity by bio-assay							
3	SoCo	SoCo	S ₄ Co	SoCo	SoCo	SoCo	S ₄ Co	SoCo
4	SoC ₈	SoC ₃₂	S ₃₂ Co	S ₃₂ Co	S ₄ Co	S ₄ Co	S ₈ Co	S ₄ Co
5	S ₁₆ C ₆₄	S ₈ C ₁₂₈	S ₁₆ Co	S ₃₂ Co	S ₁₆ Co	S ₈ Co	S ₁₆ Co	S ₁₆ Co
6	S ₆₄ C ₅₁₂	S ₃₂ C ₁₀₂₄	S ₁₆ Co	S ₃₂ Co	S ₃₂ Co	S ₃₂ Co	S ₆₄ Co	S ₃₂ C ₆₄
7	S ₃₂ C ₁₀₂₄	S ₃₂ C ₁₀₂₄	S ₃₂ Co	S ₁₆ Co	S ₆₄ Co	S ₃₂ Co	S ₆₄ Co	S ₈ C ₁₂₈
10	S ₃₂ C ₁₀₂₄	S ₃₂ C ₁₀₂₄	S ₃₂ Co	S ₃₂ Co	S ₃₂ Co	S ₃₂ Co	S ₃₂ Co	S ₁₆ C ₆₄
13	S ₃₂ C ₈₁₉₂	S ₃₂ C ₅₁₂	S ₁₆ Co	S ₃₂ C ₆₄	S ₃₂ Co	S ₃₂ Co	S ₆₄ C ₆₄	S ₃₂ C ₅₁₂
17	S ₃₂ C ₄₀₉₆	S ₆₄ C ₅₁₂	S ₁₆ Co	S ₁₆ C ₁₂₈	S ₁₆ C ₆₄	S ₁₆ C ₂₅₆	S ₆₄ C ₅₁₂	S ₁₆ C ₂₅₆
24	S ₈ C ₂₀₄₈	S ₈ C ₂₅₆	S ₈ C ₁₂₈	S ₁₆ C ₅₁₂	S ₈ C ₂₅₆	S ₄ C ₁₂₈	S ₈ C ₅₁₂	S ₃₂ C ₅₁₂
	Drift of pH							
3	4.9	5.1	2.6	2.9	3.1	3.7	3.9	4.0
4	4.2	4.0	2.6	3.0	4.3	4.1	4.1	4.3
5	3.8	3.7	2.6	2.9	4.3	4.2	5.1	4.4
6	3.6	3.6	2.6	2.9	4.6	4.2	4.6	4.2
7	3.4	3.5	2.5	2.9	4.6	4.3	4.7	4.3
10	3.5	3.5	2.6	3.0	4.4	4.1	4.5	4.2
13	3.6	3.7	2.7	3.1	4.3	4.3	4.5	4.2
17	3.7	3.7	2.7	3.1	4.4	4.2	4.5	4.3
24	3.8	3.8	2.8	3.1	4.1	4.0	4.2	4.2

TP > *T* > *ANP* > *AN* > *NP* > *N*. Sporulation started within the next few days appearing earliest on *T* and *TP* and last on *N* and *NP*. After six days' growth most felts were sporulating vigorously, the conidial masses being a dull sage green in colour. Thereafter the conidial masses became progressively more grey and at the close of the experiment little green colour remained.

The assay figures in Table 2 show clearly that potassium nitrate nitrogen was best for development of 'curling factor' activity, since activity developed rapidly and a higher peak assay was reached than on other media. The higher phosphate level led to an earlier development of activity but a lower peak level of activity. The next best medium was that with tryptone nitrogen and high phosphate level (*TP*) followed by the corresponding medium with low phosphate. These were much inferior to potassium nitrate media. The media with nitrogen presented as ammonium nitrate

or tartrate showed no development of typical 'curling factor' activity until after seventeen days' incubation, as compared with only four days with nitrate nitrogen. The ammonium nitrogen cultures did show signs of early production of some inhibiting factor as indicated in Table 2 by recording 'stunting' at several dilutions without 'curling'.

After initial fluctuations a steady pH was maintained on all media. This was very low on the ammonium tartrate media and almost as low on the nitrate media, suggesting production of an organic acid by the mould.

Organic supplements

In some earlier experiments it was found that addition of 0.1 % peptone or 0.01 % yeast extract to Czapek-Dox medium stimulated the development of 'curling factor' activity. Further detailed investigation failed to confirm that any consistent benefit could be obtained from such additions, or from addition of various vitamins (aneurin hydrochloride, nicotinic acid, riboflavin or pyridoxin) or amino-acids (*dl*-alanine, glycine, *l*-tryptophane, β -phenylalanine, *l*-tyrosine, *p*-aminobenzoic acid or glutamic acid). Addition of peptone, or yeast extract, does sometimes lead to a more rapid establishment of a confluent mycelial felt and in production experiments (see next section) 0.1 % peptone was added to all media as a routine measure.

ISOLATION OF 'CURLING FACTOR'

'Curling factor' is thermostable; it was demonstrated that culture filtrates showing 'curling factor' activity could be boiled for thirty minutes or autoclaved for twenty minutes without loss of activity. It was even found possible to evaporate culture filtrates to dryness in an open dish without appreciable loss in activity, but the dark brown gum, containing caramelized sugars, produced in this manner did not seem a suitable starting point for fractionation and other methods of concentration of the 'curling factor' were investigated.

Preliminary experiments indicated that the 'curling factor' could be removed from active culture filtrates by extraction with chloroform, *n*-butyl alcohol or ether, or by treatment with activated charcoal. Extraction of culture filtrates with ether or *n*-butyl alcohol has its disadvantages; if ether is used much solvent is lost, owing to the water solubility of ether and *n*-butyl alcohol extracts more coloured material from the medium than the other solvents. Accordingly further work was concentrated upon methods of extraction utilizing chloroform or activated charcoal.

The results of an experiment on methods of extraction are shown in Table 3. Here *Penicillium Janczewskii* was grown at 25° C. on 500 ml. lots of medium in flat earthenware vessels of the type used for production of penicillin by Abraham *et al.* (1941). The media used were Raulin-Thom (5 % dextrose) and Czapek-Dox (5 % dextrose), both supplemented with 0.1 % peptone. Samples were taken after twelve days' growth. The extraction methods examined were (*a*) chloroform extraction of the culture filtrate, and (*b*) treatment of the culture filtrate with activated charcoal

followed by elution of the charcoal with chloroform or ether. Each of these methods is shown to be effective, whether judged by removal of 'curling factor' activity from the culture filtrate or by the activity of the material extracted. As a result of this and other similar experiments it was decided to concentrate in further work on chloroform extraction of culture filtrates.

After sixteen days' growth the cultures used for the extraction experiment just described were harvested and the culture filtrates extracted with chloroform. On evaporation of the chloroform at reduced pressure a yellowish gum with some crystalline material was obtained from the Raulin-Thom cultures, and a reddish-orange gum from the Czapek-Dox cultures. These gums were taken up in a little hot ethyl alcohol; on cooling pale straw-coloured crystals separated from the Raulin-Thom material and a reddish, largely amorphous powder from the Czapek-Dox material.

Table 3. *Activity by bio-assay of fractions obtained from Penicillium Janczewskii culture filtrates by various extraction procedures*

Fraction assayed	Medium	
	Czapek-Dox + peptone	Raulin-Thom + peptone
1. Untreated culture filtrate	S ₁₆ C ₅₁₂	S ₃₂ C ₂₅₆
2. Culture filtrate after chloroform extraction	So Co	So Co
3. Chloroform extract made up to original volume	S ₁₆ C ₅₁₂	S ₁₆ C ₁₂₈
4. Culture filtrate after treatment with charcoal	So Co	So Co
5. Chloroform eluate from charcoal made up to original volume	S ₁₆ C ₅₁₂	S ₈ C ₁₂₈
6. Ether eluate from charcoal made up to original volume	S ₈ C ₅₁₂	S ₈ C ₆₄

The yield of the former was 23 mg./l. of culture filtrate, of the latter 81 mg./l. Both these materials, when dissolved in water, showed by assay curling activity at a minimum concentration of 0.8 µg./ml. Preparations obtained by this process are described below as crude 'curling factor'.

Table 4 shows data for a number of batches of cultures run for production purposes in earthenware culture vessels. Four general conclusions may be drawn from these results:

(a) Czapek-Dox is a more favourable medium for production of 'curling factor' than Raulin-Thom; this is in general agreement with the results presented in Table 2, which indicated that nitrate nitrogen was more favourable than ammonia nitrogen for production of 'curling factor'.

(b) Increase in the sugar content of Czapek-Dox from 5.0 % to 7.5 % has resulted in increased yields of crude 'curling factor'. The colour of the product is also considerably improved; in these high glucose batches the crude 'curling factor' extracted was of a relatively high degree of purity.

(c) Increase in the sugar content of Czapek-Dox to 7.5 % or higher markedly affects the pH drift; the medium maintains an almost steady pH with 7.5 % glucose, as compared with a steady upward drift with 5.0 % glucose. With 12.5 % glucose the medium actually becomes more acid. These results like those from Table 2, previously discussed, suggest that in

high glucose media *Penicillium Janczewskii* produces considerable quantities of an organic acid. The effect of increasing glucose on pH may be responsible for the increased quantity and purity of yields of 'curling factor'.

(d) The activity of the culture filtrate as determined by bio-assay roughly follows the yields of crude 'curling factor'.

EFFECT OF PURE 'CURLING FACTOR' ON *BOTRYTIS ALLII*

A sample of pure 'curling factor', prepared from the crude product by recrystallization from a mixture of equal quantities of diethylene dioxide

Table 4. Production of 'curling factor': yield data

Batch no.	Medium	Glucose %	Period of incubation (days)	pH of culture filtrate	Assay of culture filtrate	Yield of crude 'curling factor' (mg./l.)	Appearance of crude product
42	RT	5.0	16	—	S ₁₆ C ₁₂₈	23	Yellow-brown crystals
56	RT*	5.0	18	5.0	SoCo	Neg.	Grey powder
111 A	RT	5.0	14	7.2	S ₈ C ₆₄	Neg.	Grey powder
117	RT	5.0	12	4.1	S ₁₆ C ₂₅₆	23	Yellow-brown crystals
121	RT	5.0	15	3.5	S ₁₆ C ₁₂₈	9	Yellow-brown crystals
149 C	RT	10.0	17	2.9	S ₁₆ C ₂₅₆	37	Pale brown crystals
47	CD	5.0	15	7.3	S ₈ C ₅₁₂	81	Reddish powder
57	CD*	5.0	18	7.3	S ₄ C ₂₅₆	24	Brown crystals
111 B	CD	5.0	14	7.9	S ₄ C ₆₄	8	Brown powder
118	CD	5.0	16	7.2	S ₁₆ C ₁₂₈	48	Yellow-brown crystals
134 A	CD	7.5	16	4.8	S ₃₂ C ₅₁₂	155	Yellow crystals
134 B	CD	7.5	20	6.0	S ₁₆ C ₅₁₂	82	Pale brown crystals
142 A	CD	7.5	14	3.6	S ₁₂₈ C ₂₀₄₈	151	Yellow crystals
149 A	CD	7.5	12	3.8	S ₅₁₂ C ₂₀₄₈	166	Pale yellow crystals
154 A	CD*	7.5	18	4.2	S ₁₂₈ C ₅₁₂	124	Brownish yellow crystals
154 C	CD	7.5	14	3.6	S ₃₂ C ₁₀₂₄	65	Brownish yellow crystals
149 B	CD	10.0	14	3.6	S ₁₂₈ C ₄₀₉₆	158	Yellow crystals
142 B	CD	12.5	22	3.5	S ₃₂ C ₅₁₂	59	Yellow crystals
154 B	CD	12.5	18	3.5	S ₃₂ C ₁₀₂₄	51	Pale brown crystals

* 1000 ml. medium, per culture vessel (all others 500 ml. per vessel).

RT=Raulin Thom+peptone. CD=Czapek-Dox+peptone. Neg.=negligible yield.

(dioxan) and water, as recommended by McGowan (1946), was dissolved in half strength Weindling solution and assayed by the *Botrytis Allii* spore germination technique already described. The effect produced was similar to that produced by active *Penicillium Janczewskii* culture filtrates, as may be seen by comparison of Fig. 2 (dilutions of pure 'curling factor') with Fig. 1 (dilutions of an active culture filtrate). From Fig. 2 it is apparent that concentrations of the order of 2.5 µg./ml. of 'curling factor' produced marked stunting and distortion of germ tubes and that the characteristic 'waving' of hyphae is clearly visible in concentrations of 0.16 µg./ml. It was not visible at 0.08 µg./ml.

These observations may be used to check the correlation between bio-assay of culture filtrates and yield of 'curling factor'. In Table 5 the yields from the batches dealt with previously in Table 4 are compared with an 'expected yield', calculated by assuming (on the basis of the assay of pure, crystalline 'curling factor') that in the greatest dilution of culture filtrate showing waving of the hyphae there is a concentration of 0.16 µg./ml. of

'curling factor'. Considering the known inaccuracies of the assay method the actual yield follows the expected yield reasonably closely.

The effect of 'curling factor' on the germination of *Botrytis Allii* conidia can now be considered in greater detail. By observation of germination drops over a period of four days, it was found that in concentrations of 20 µg./ml. 'curling factor', a markedly stunted germ-tube is produced (see Fig. 2 A) and that growth ceases at that stage. In higher concentrations (e.g. 100 µg./ml.) germination may be a little delayed but almost complete germination does occur, growth ceasing once the short swollen germ-tube is produced.

Table 5. *Correlation between bio-assay and yield*

Batch no.	Activity by bio-assay	'Expected yield' (mg./l.)	Yield found (mg./l.)
42	S ₁₆ C ₁₂₈	20	23
56	So Co	—	—
111 A	S ₈ C ₆₄	10	—
117	S ₁₆ C ₂₅₆	41	23
121	S ₁₆ C ₁₂₈	20	9
149 C	S ₁₆ C ₂₅₆	41	37
47	S ₈ C ₅₁₂	82	81
57	S ₄ C ₂₅₆	41	24
111 B	S ₄ C ₆₄	10	8
118	S ₁₆ C ₁₂₈	20	48
134 A	S ₃₂ C ₅₁₂	82	155
134 B	S ₁₆ C ₅₁₂	82	82
142 A	S ₁₂₈ C ₂₀₄₈	327	151
149 A	S ₅₁₂ C ₂₀₄₈	327	166
154 A	S ₁₂₈ C ₅₁₂	82	124
154 C	S ₃₂ C ₁₀₂₄	164	65
149 B	S ₁₂₈ C ₄₀₉₆	654	158
142 B	S ₃₂ C ₅₁₂	82	59
154 B	S ₃₂ C ₁₀₂₄	164	51

When included in a Czapek-Dox agar medium the effect of 'curling factor' on germination and growth of *Botrytis Allii* is similar. At a concentration of 1 µg./ml. the colonies are much reduced in size (a diameter of 2 cm. after four days' growth at 25° C. as compared with 6 cm. in control), the rate of radial spread being much reduced. The advancing hyphae at the edge of such colonies are much waved, contorted and excessively branched. With concentrations of 10 µg./ml. and 25 µg./ml. no growth was visible to the naked eye, but examination of plate cultures microscopically showed that all spores had germinated, producing germ-tubes similar to those illustrated in Fig. 2 A-D; observations after seven days showed that no further development had taken place. If these germinated spores were then removed to normal Czapek-Dox agar, growth proceeded more or less normally.

Thus the physiological effects of 'curling factor' on *B. Allii* may be summarized as follows:

(a) In low concentrations (1 µg./ml. or less) growth is retarded, and the hyphae produced are excessively branched, waved and contorted.

(b) In higher concentrations (5-100 µg./ml.) germination of conidia is not prevented but growth ceases after a certain early stage of development of the germ-tube is reached, although death does not immediately occur.

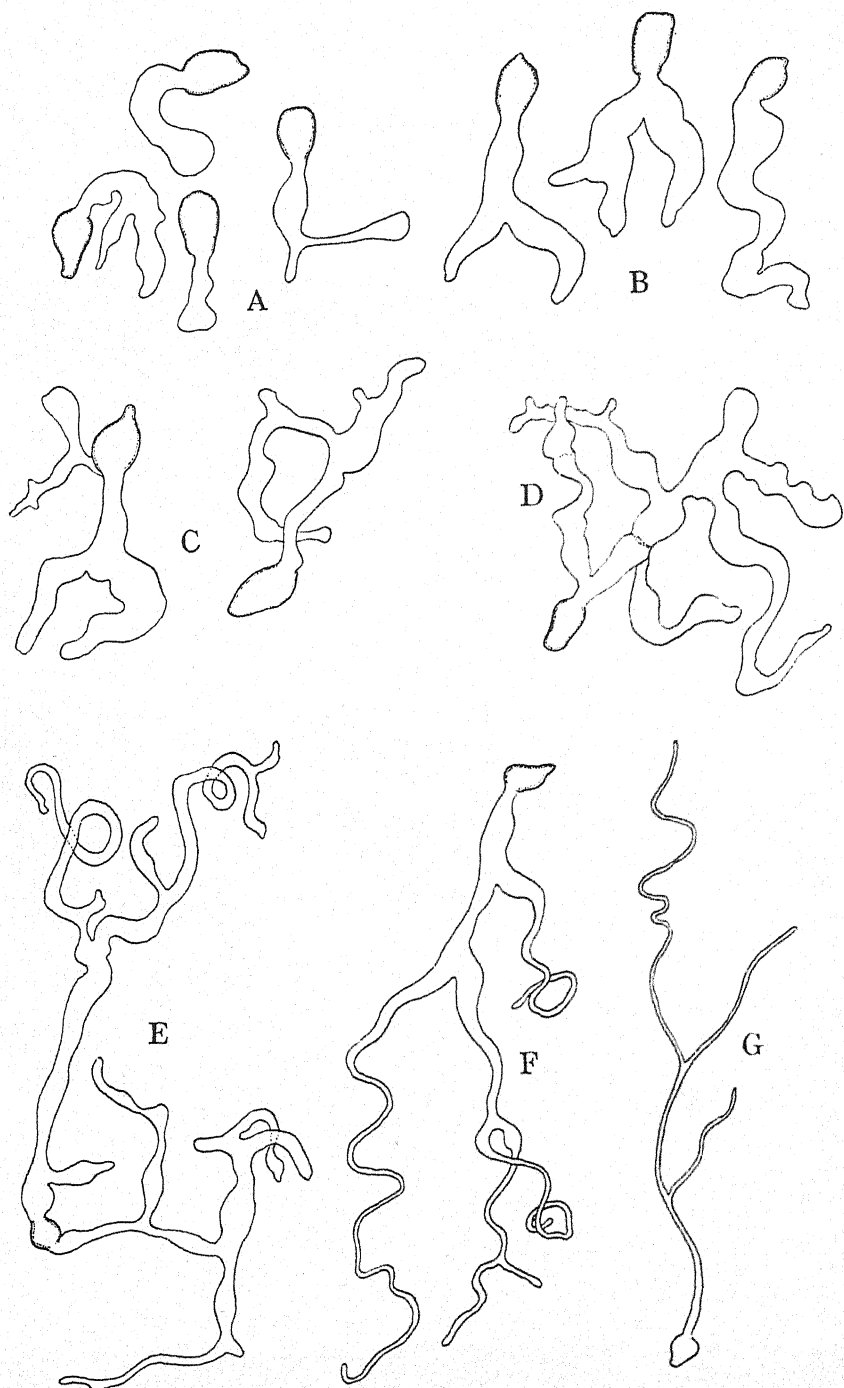


Fig. 2. Effect of various concentrations of pure crystalline 'curling factor' on germination of *Botrytis Allii*. Concentrations: A, 20 $\mu\text{g./ml.}$; B, 10 $\mu\text{g./ml.}$; C, 5 $\mu\text{g./ml.}$; D, 2.5 $\mu\text{g./ml.}$; E, 1.25 $\mu\text{g./ml.}$; F, 0.625 $\mu\text{g./ml.}$; G, 0.16 $\mu\text{g./ml.}$ (A-F $\times 1.5$; G $\times 1$.)

Preliminary observations indicate that the effect of 'curling factor' on other fungi is qualitatively similar. No effects on growth of bacteria have been observed.

DISCUSSION

The two most interesting physiological effects of 'curling factor' on *Botrytis Allii* are (a) inhibition of growth only at a stage subsequent to germination of the conidia and (b) the remarkable increase in branching and distortion of hyphae produced by low concentrations.

The use of the terms 'fungistatic' and 'fungicidal' has been discussed by McCallan & Wellman (1942), who point out that whereas ideally a fungistatic property may be defined as the ability to prevent growth of fungi without killing and a fungicidal property as the power to kill fungal spores or mycelium, in practice it is not possible to disentangle the two properties completely. A substance which is fungicidal at a given concentration is also fungistatic. However, one can in general terms distinguish between fungicidal substances, which actually kill fungi at concentrations close to those which merely produce a fungistatic or inhibitory effect, and fungistatic substances which prevent growth of fungi at concentrations well below that needed to cause a rapid killing effect. The distinction is probably concerned with the degree of reversibility of the inhibitory mechanism. 'Curling factor' is a typically fungistatic substance, using the term in the sense described above, but of an unusual type. It is fungistatic in so far as growth is markedly restricted at concentrations well below those needed to prevent germination or permanently to stop growth, but its effect is unusual in that the inhibition of growth is most marked at a stage subsequent to germination.

The effects of 'curling factor' on the growth form of *B. Allii* hyphae are unique. Many substances which have fungicidal or fungistatic activity may produce stunted and slightly distorted growth forms at concentrations just below that required to cause inhibition of growth. Boric acid is an example. 'Curling factor' is remarkable in that easily detectable effects on growth form are produced at concentrations far below that required to arrest growth. The distortion and excessive branching which it causes are of a much more pronounced type than any previously described as being caused by pure chemicals.

Similar morphological effects on fungi, caused by metabolic products of micro-organisms, have been noticed before. Porter (1924) has described the distortion of hyphae of species of *Helminthosporium* when growing near to colonies of a *Bacterium*. His description is very reminiscent of the effects of 'curling factor'—'when the hyphae of *Helminthosporium* are within 2 cm. of the bacterial colony, growth slackens and eventually ceases. Branches are given off from the hyphae in every direction and these become gnarled and twisted, and bubble-like enlargements of varying size appear in this portion of the mycelium'. Elliot (1917) has described distortions of species of *Alternaria* grown in the proximity of an unidentified *Bacterium*. Cook, Kreke, Giersch and Schroeder (1941) have prepared concentrates from yeast which, included in culture media, cause the formation of thick,

gnarled mycelia by fungi. Reinhardt (1892) and Buller (1933) illustrate a distortion and irregular branching of hyphae of *Sclerotinia sclerotiorum* when grown in the proximity of *Mucor*. It seems probable that substances causing abnormal morphological development of fungal hyphae are produced by a wide variety of micro-organisms.

SUMMARY

Strains of *Penicillium Janczewskii* Zal. are shown to produce a substance, when grown on synthetic liquid culture media, which causes an unusual stunting and distortion of the germ-tubes and hyphae of *Botrytis Allii* and other fungi.

This substance, known as 'curling factor', is produced more abundantly in media containing nitrogen as nitrate than in media containing nitrogen as ammonium salts or peptone. The optimum concentration of glucose in the media is 7.5 %, which is noticeably better than 5.0 %. Increase in glucose concentration to 10.0 % or higher gives no further advantage.

The 'curling factor' may be removed from active culture-filtrates by extraction with chloroform, ether or *n*-butyl alcohol or by treatment with activated charcoal followed by elution of the charcoal with ether or chloroform. By extraction with chloroform followed by successive crystallization from ethyl alcohol and dioxan/water mixtures, 'curling factor' has been obtained in pure form as colourless crystals. Yields of 150 mg./l. of culture filtrate can be obtained.

In concentrations of 25 µg./ml., 'curling factor' causes the production of short stunted germ-tubes by *Botrytis Allii*, whose development ceases at an early stage; lower concentrations (1.0 µg./ml.) cause excessive branching and distortion and lower concentrations still (0.2 µg./ml.) cause a waving of the hyphae. It is characteristic of the substance that relatively high concentrations (100 µg./ml.) do not prevent germination of *B. Allii* conidia, though far lower concentrations cause the profound physiological effects described.

We are much indebted to Miss Myrtle Bray for assistance with the bio-assays and to Mr George Elson for help in production and isolation of 'curling factor'.

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(Accepted for publication 3 June 1946)

A SUBSTANCE CAUSING ABNORMAL DEVELOPMENT OF FUNGAL HYPHAE PRODUCED BY *PENICILLIUM JANCZEWSKII* ZAL.

II. PRELIMINARY NOTES ON THE CHEMICAL AND PHYSICAL PROPERTIES OF 'CURLING FACTOR'

By J. C. MCGOWAN, *Hawthorndale Laboratories, Jealott's Hill Research Station*

In the preceding paper (Brian, Curtis & Hemming, 1946) an account is given of the isolation and production of a metabolic product of *Penicillium Janczewskii*, 'curling factor', which in low concentrations causes unusual stunting and distortion of germ-tubes and hyphae of *Botrytis Allii* and other fungi. The crude substance could not as a rule be satisfactorily purified by repeated crystallization from ethyl alcohol. However, after recrystallization from either aqueous 1:4-dioxane, or isobutyl alcohol, or aqueous tetrahydrofurfuryl alcohol and then from ethyl alcohol, colourless octahedral crystals were obtained. The melting point was 220° C. 'Curling factor' contained no nitrogen, no sulphur, and no halogens. Found: C, 59.3, 59.2, and 58.5 %; H, 4.9, 5.0 and 4.9 %; and mol. wt. (Rasts' method), 398, 387 and 378. $C_{20}H_{20}O_9$ requires: C, 59.4 %; H, 5.0 %; and mol. wt. 404.

'Curling factor' is optically active and the specific rotation $[\alpha]_D^{17}$ observed by sodium D light at 17° C., using a 2 % solution in chloroform, had the very high value of +370°.

The pure 'curling factor' is very soluble in acetic acid, 1:4-dioxane, and benzene, is soluble in ether and ethyl alcohol and is only slightly soluble in ligroin. The octahedral crystals described above dissolve readily in cold chloroform but crystallization soon sets in and needle-shaped crystals separate. 'Curling factor' is only sparingly soluble in hot carbon tetrachloride and separates in needles when solutions in this solvent are cooled.

There are three methoxyl groups in each molecule of 'curling factor'. One methyl group attached to carbon is indicated and hydroxyl groups seem to be absent. Work on the chemical constitution is in progress.

The author wishes to thank Mr P. J. Curtis for preparation of the 'curling factor' with which to do this work.

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- BRIAN, P. W., CURTIS, P. J. & HEMMING, H. G. (1946). A substance causing abnormal development of Fungal Hyphae produced by *Penicillium Janczewskii* Zal. I. Biological assay, production and isolation of 'curling factor'. *Trans. Brit. mycol. Soc.* xxix, 173-87.

(Accepted for publication 3 June 1946)

PROCEEDINGS

Meeting held in the Department of Biology, Chelsea Polytechnic, London, at 11.15 a.m., 23 February 1946. The President, Dr J. Ramsbottom, in the Chair.

G. C. AINSWORTH. Notes on the taxonomy and nomenclature of British Smuts.

Miss K. SAMPSON. Notes on the biology of British Smuts.

O. DE ROUSSET-HALL. Exhibit of agarics preserved by freeze-drying.

E. A. ELLIS. *Symphyosira* Preuss. and two British Discomycetes with *Symphyosira*-like conidial organs.

P. W. BRIAN. Fungistatic substances produced by *Trichoderma viride*.

E. W. MASON. The name *Helminthosporium velutinum*.

S. J. HUGHES. Some specimens called *Helminthosporium velutinum*.

Meeting held in the Board Room of the British Museum (Natural History), South Kensington, London, from 2 to 4 p.m., 12 April 1946. At 2.30 p.m., after a short business meeting, the President commented on a series of

Exhibits illustrating the development of British Mycology and the history of the Society

from the Museum collections and the Society's archives. The exhibits included the Berkeley and Browne correspondence, original drawings of fungi by noted botanical artists, a virtually complete series of mycological books published in this country during the past fifty years, photographs of past-Presidents, and a number of old foray photographs.

Meeting held in the Department of Botany, Glasgow University, at 10.30 a.m., 28 June 1946. The Chairman of the Plant Pathology Committee, Mr W. C. Moore, in the Chair.

A. E. W. BOYD, C. E. FOISTER and A. R. WILSON. Infestation of soils by *Fusarium caeruleum* in relation to the incidence of dry rot in potatoes.

Miss ELIZABETH GRAY. Problems in plant pathology in the North of Scotland.

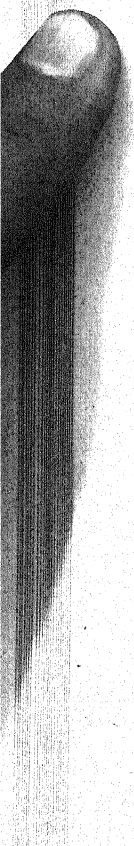
C. H. CADMAN. Raspberry pathology in Scotland.

H. F. DOVASTON. The ascigerous stage of a species of *Epicoccum*.

Miss MARY NOBLE. A brief history of early mycological work in Scotland.

MALCOLM WILSON. Alpine rusts in Scotland.

Mrs ELSIE CONWAY. Exhibit of Japanese and British agars.



NEW RECORDS AND OBSERVATIONS. III

By A. A. PEARSON, F.L.S.

(With Plates IX-XII)

Most of the species recorded in the present paper were collected during the war years. They recall many pleasant forays in the woods of Surrey, Sussex and Hampshire within easy reach of Haslemere, in the genial company of my friend Mr E. W. Swanton. During these years of grim memories, we were collecting for medical research, and some of the first results of our efforts may be read in the paper by Drs W. H. Wilkins and G. C. M. Harris in the *Annals of Applied Biology*, vol. xxxi, pp. 262-70.

Other species are included which were sent by correspondents from various parts of Britain, whose activities are much appreciated though sometimes too overwhelming for comfort. One particularly good hunting ground proved to be Braunton Burrows, North Devon, already famous for its flowering plants.

I am much indebted to Miss E. M. Wakefield and Dr R. W. G. Dennis for so successfully portraying the four species on the two coloured plates, and for their help and criticism. Thanks are also due to Major Maxwell Knight, Mr F. Ballard and Mr Colin G. Fletcher for their excellent photographs. In work of this kind, it is indispensable to consult mycological books that are to be found in few libraries, and I must express my gratitude at being allowed the free use of the library at the Herbarium, Royal Botanic Gardens, Kew.

***Amanita pantherina* DC. *forma robusta* n.f.**

Pileus 8-9 cm. wide, convex, slightly gibbous, fleshy, dull brown (tawny olive, Ridgway) with darker streaks, viscid, polished, smooth, with remains of white veil in rather large irregular patches adhering to surface and appendiculate to the margin which is incurved and more or less striate. *Gills* free, crowded, broadly linear or arcuate, blunt in front, narrowed behind, white, edge smooth or slightly fimbriate. *Stem* 10 cm. long, 1.5-2 cm. diam., attenuated above, firm, solid then hollow, white, fibrillose below, with clear-cut marginate bulb; one ring half-way up, another just above bulb which shows further traces of ring markings. *Flesh* white, unchangeable. *Taste* mild. *Smell* none when fresh. *Spore powder* white. *Spores* non-amyloid, broadly elliptical with granular contents 11-13 x 8.9 μ .

Habitat: open space of wood in grass near birches, on chalk soil. Near East Dean, Sussex, 22 September 1943. Numerous specimens present.

This handsome, thick-set agaric is so different from the relatively slender type which in Britain has been associated with this species that it

deserves a distinctive name. For the present it should suffice to call it forma *robusta*. A very closely allied form, equally robust, has been described by Gilbert as *Amanita abietum* (Konrad & Maublanc, *Ic. Sel. Fung.* 1, Pl. 7 (1926)), which differs from the above in its habitat under conifers and the non-striate margin of the pileus.

Lepiota pseudo-felina Lange, *Dansk Bot. Ark.* ix, no. 6, 63 (1938) and *Fl. Ag. Dan.* 1, 30, Pl. 12, fig. C (1935).

Pileus 1–2.5 cm. fleshy in centre, campanulate then spreading with prominent obtuse umbo, brownish black in centre, thinning out towards margin into small blackish squamules, showing white flesh underneath. *Gills* not crowded, ventricose, free, white, edge smooth or fimbriate. *Stem* white with striate fibrils, rufescent, sheathed two-thirds up and with scattered small black scales but no well-formed ring. *Flesh* white in pileus and in the pith-like centre of stem, horny brown at the sides. *Smell* mealy. *Taste* mild but rather rancid. *Spore powder* white. *Spores* amyloid, turning reddish in iodine, truncate, spurred, mostly $8.5 \times 3.5 \mu$ (Lange's measurements $9-10.75 \times 3.25-4 \mu$). *Gill edge* sterile with closely packed cylindrical or ventricose rather thick-walled *cystidia*, often in bunches $8-9 \mu$ wide. *Hairs on cap* obtuse or lanceolate of different lengths $10-16 \mu$ wide, black or only faintly tinted grey.

Habitat: in a troop under elm trees, 22 November 1945. Sent by Dr F. R. Elliston Wright from Braunton, N. Devon.

Our specimens were larger and more robust than *L. felina* (Pers.) Fr., unlike the smaller solitary specimen originally found by Lange. All parts have a tendency to turn a salmon tint when rubbed.

Tricholoma ionides (Bull.) Fr. var. **obscurissima** n.var.

Pileus 3–7 cm. shallow, convex, mat, smooth or somewhat rugulose, fuliginous-violaceous, margin incurved at first, sometimes undulate. *Gills* crowded, pale buff, emarginate, linear or ventricose, edge smooth. *Stem* 4–6 cm. long, 5–7 mm. thick, slightly paler than cap, striate, equal or with bulbous base, solid or hollow, white tomentum at base. *Smell* none. *Taste* rancid. *Flesh* horny colour. *Spores* in mass white, elongate-elliptical $5-6 \times 3-3.5 \mu$ with or without guttules, non-amyloid. *Cystidia* absent.

Habitat: in troops under pine trees, November 1944 and 1945. Witley Common, Surrey. It is much the colour of the pine needles and easily overlooked. At first sight it may be taken for *Tricholoma melaleucum* and has probably been passed over as a form of this protean species. It was seen in troops of a dozen or so, but not caespitose.

LATIN DIAGNOSIS. *Tricholoma ionides* (Bull.) Fr. var. *obscurissima* Pearson. *A typo differt colore pilei et stipitis violaceo-fuligineo; colore lamellarum pallidoluteolo; etiam caro fusca; caetera concordant.*

Mycena fellea Lange.

In a previous paper of this series (*Trans. Brit. myc. Soc.* xxvi, 39) I used this binomial rather than *M. erubescens* v. Höhnelt, but I have since received a very interesting note from M. Marcel Josseland who writes that he was

once in a district where this species grew abundantly and he had the chance of seeing tens of thousands of examples growing on the mossy trunks of conifers. He found it extremely polymorphous, varying in size from 8 to 32 mm., often with a white milk as in *M. galopus*, and sometimes turning a pink or even a bright carmine.

All this confirms the opinion of Kühner that *M. fellea* is identical with *M. erubescens*, and as the epithet *erubescens* was used a year before Lange introduced his *M. fellea*, we have to accept it.

Mycena erubescens v. Höhnelt = *M. fellea* Lange.

To the description given add: sometimes exuding white milk and turning reddish.

Mycena quisquiliaris (Joss.) Kühner in *Le Genre Mycena*, 388 (1938) = *Omphalia quisquiliaris* Jossierand, *An. Soc. Linn. Lyon*, LXXX, 88 (1937).

Pileus usually very small, full range 1-10 mm. membranaceous, white, fragile, convex or umbilicate, rarely papillate, when old sulcate with crenulate margin, dry, pubescent. *Gills* few, distant but not pliciform, white, adnate, linear or arcuate, edge denticulate seen under strong lens. *Stem* very thin thread-like, usually short, straight or curved, hyaline-white, pubescent, base often with minute pubescent bulb. *Basidia* four-spored. *Spores* amyloid; obovate, tear-shaped or subfusiform, smooth 11-13 × 4-6 μ. *Cystidia* on gill edge ventricose with blunt apex often capitate and sometimes forked 30-70 × 7-13 μ. *Cuticle* of pileus and stem with numerous cylindrical capitate or subglobose cells.

Habitat: in troops on dead debris of marsh plants especially of *Molinia caerulea*, but also recorded on *Carex* sp. and *Rubus*. Collected by Mr E. A. Ellis on *Cladium* at Wheatfen, December 1942.

This small agaric is probably very common. It is said sometimes to occur in vast numbers on *Molinia*. Doubtless it has escaped being recorded on account of its small size and fragility. The cap may be as small as a pin's head, but larger specimens occur and in a glass tube will keep for days. Specimens will usually be found not on the surface but underneath a pile of debris where the moist atmosphere enables it to flourish.

Mycena arcangeliana Bres. var. *Oortiana* Kühner, *Le Genre Mycena*, 297 (1938). *De Nederl. Myc. A. S. P. Oort*, 229 (1928).

Pileus 1-1.5 cm. obtusely conical, at first greyish brown, then white with a very slight olive tint, striate sulcate half-way to centre. *Gills* crowded, adnexed, attenuated towards stem, white then pinkish, edge fimbriate. *Stem* 2-4 cm. long, 1-1.5 mm. thick, cylindrical, polished, lilaceous grey, darker when young, tomentose at base, hollow. *Flesh* white in cap, grey in stem. *Smell* strong of iodoform. *Taste* mild. *Basidia* four-spored. *Gill edge* sterile with closely packed club-shaped or pyriform warted cystidia mostly 12-14 μ wide. *Spore powder* white. *Spores* elliptical 7-8 × 4.5-5 μ one guttulate, smooth, amyloid.

Habitat: caespitose on rotting elder (*Sambucus Ebulus*). Collected by Dr R. W. G. Dennis at Mickleham, Surrey, 15 October 1945. On Pl. IX D, Dr Dennis has provided an excellent drawing of his specimens.

The above description corresponds closely to that given by Oort, his range of size being larger: pileus 1-2.5 cm., stem 3.5-10 cm. long, 1-3 mm. thick. He recorded his specimens as *Mycena arcangeliana* Bres., but Kühner made it into a variety though he might well have given it specific rank, since it differs in many respects from the description given by Bresadola in *Bull. Soc. bot. it.* 78 (1904). The smell in the type species is strong '*fere carnis assatae*' which Kühner translates as 'chair brûlée', though it may well mean roasted meat rather than burnt flesh; the habitat is on a palm stem.

The iodoform smell and warted cystidia place *M. Oortiana* in a well-defined group of *Mycena*, the commonest species of which we usually call *M. metata*, but which Kühner effectually disguises under the name of *M. vitrea* var. *tenella*.

Omphalia Mairei Gilbert, *Bull. Soc. Myc. Fr.* XLII, 63 (1926) and Jossierand, *Soc. Linn. Lyon*, LXXX, 14 (1937).

Pileus 5-15 mm. thin, convex soon expanding, striate by transparence, whitish or greyish hyaline. *Gills* few and distant, fairly wide and rather thick, sometimes anastomosing, arcuate, decurrent or subdecurrent, edge blunt and smooth. *Stem* filiform 2-3 cm. long, equal or thickened above, white hyaline, smooth. *Smell* of new meal or none. *Taste* mild. *Basidia* with four sterigmata. *Cystidia* absent. *Spores* in mass, white, boat-shaped or virguliform, usually pointed at both ends, but some are round at one end, with or without guttule, mostly $8 \times 4.5 \mu$ in specimens examined ($7-9 \times 3-4 \mu$ Gilbert. $8-9.5 \times 4-4.8 \mu$ Jossierand), non-amyloid.

Habitat: in quantity on bank of wet ditch. Haslemere, August and October 1944. Also by Dr F. N. Elliston Wright in sandy soil among dunes, Branton Burrows, 30 October 1944. Probably common.

Two other species of *Omphalia* have spores similar to the above: *O. integralla* Fr. has narrow, often vein-like gills and amyloid spores. *O. candida* Bres. is caespitose on dead rhizomes of comfrey, has a long stem and deeply decurrent gills.

Marasmius oreadoides (Pass.) Fr., Passerini, *Fungi Parmensi*, in *Nuovo Gior. Bot. It.*, 109 (1872). Saccardo, *Fl. It. Crypt.* Pars 1, *Fungi*, 470.

Pileus 2-5 cm. densely caespitose, somewhat fleshy, convex or flat but very irregular in outline with contorted folds, smooth, mat, whitish or pinkish buff with brick-coloured patches when old, margin incurved at first. *Gills* distant, white or cream, of various shapes and sizes, broad or narrow, linear or ventricose, mostly free. *Stem* 3-5 cm. long, 4-10 mm. wide, confluent with pileus, compressed, striate with silky pruinose fibrils, white, hollow, mostly with acuminate base and often with white tomentum at base. All parts elastic. *Flesh* very pale buff. *Smell* pleasant. *Taste* rather bitter after mastication. *Spores* white in mass, subfusiform (cylindric apiculate) $6-6.5 \times 2.5-3 \mu$. *Cystidia* absent.

Habitat: collected by Mr R. H. Johnston at Ballock Park, southern end of Loch Lomond, Scotland. Growing luxuriantly on heap of old leaves, 3 and 10 October 1944.

The only doubt I have in assigning this to *M. oreadoides* is that Saccardo gives the spores as '*ellipsoideus* $6 \times 3-3.5$ *basi vix apiculatus*' whereas our specimens had distinctly apiculate spores.

Hygrophorus (Limacium) leucophaeus (Scop.) Fr. = *H. discoideus* Quél. non Fr.

A slender species, all parts flesh-pink.

Pileus 2.5-5 cm. very viscid, fleshy in centre, flat or with small umbo, soon depressed and upturned, delicate flesh-pink, darker in centre, glabrous. *Gills* subdistant, ventricose, decurrent or adnate with decurrent tooth, pale flesh-pink. *Stem* 3-5 cm. long, 4-5 mm. thick, flexuose, usually tapering to a point, a little darker than pileus, paler above, fibrillose-floccose, solid. *Flesh* concolorous. *Taste* mild. *Smell* none. *Spores* in mass, white, elliptical $6.5-7 \times 4-5 \mu$.

Habitat: in beech woods. East Dean Park, Sussex, 30 October 1944. Elsewhere we collected a very pale form with smaller spores, but further observations are necessary to ascertain if this deserves varietal rank.

H. leucophaeus is often confused with *H. discoideus* (Pers.) Fr. which is more robust, rusty ochraceous and grows in pine woods. This latter species has been illustrated by Cooke, *Ill.* 887 (914) as *H. mesotephrus* B. & Br.

Since the above was written, we have seen vol. v of J. Lange's *Flora Agaricina Danica*, where on Pl. 163 both the typical and paler forms are illustrated, the latter being distinguished as a variety but without epithet. It can be left at that till further investigated.

Russula luteo-viridans Martin, *Bull. Soc. Bot. de Genève*, VII (1894).

Pileus 3-10 cm. wide, convex then depressed in centre, viscid, smooth except margin which is striate-tuberculate, shining greasy looking, pallid greenish buff with tinge of red towards margin; cuticle peels half-way. *Gills* crowded, edge smooth and thin, thick at base, rounded in front, attenuating to stem, attached by slight emargination, colour when mature orange buff, all one size or anastomosing at margin of pileus. *Stem* 3-5 cm. long, 3 cm. wide, cylindrical, firm at first soon soft with spongy stuffing, white, finely rugulose. *Flesh* white. *Smell* none. *Taste* rather acrid. *Chemical reactions*: Phenol: pink then dark purple. Ferrous sulphate: none at first, then greyish. *Spore powder* G. of Crawshay (light orange yellow). *Spores* amyloid, broadly ovate with long coarse warts $9.5 \times 7 \mu$ without reticulation. *Cystidia* on gill edge sparse, lanceolate, staining throughout dark blue with sulphovanillin $50-70 \times 8-11 \mu$. *Cuticle* of cap also with cystidia as above but longer and narrower than on gill.

Habitat: under oak. Jays Wood, near Gospel Green, Sussex, 9 August 1945.

This seems to be a rare species. Only one example was seen, but so exactly coinciding with the description given by J. Schaeffer in his *Russula Monographie*, p. 504, that I have no hesitation in recording it. On the Continent it has been reported as growing under spruce, fir and hornbeam. No good illustration of this species has yet been published. There is some doubt whether it is distinct from *R. urens* Romell.

Since writing the above, Romagnesi in *Bull. Soc. Myc. Fr.* LVIII, 167 (1942), has described a *R. luteo-viridans* which seems to agree with our

fungus except that no cystidia are present in the cuticle of pileus as stated above and also in Schaeffer's description. In the French specimens there are long hyphae filled with granular matter but no genuine dermatocystidia.

Pluteus Godeyi Gillet, *Champignons de France*, 395 (1874).

Pileus 1-4 cm. usually small, membranaceous, convex, slightly umbonate, bluish grey or with slight pink tinge from the gills beneath, translucently striate at margin. *Gills* subdistant, broad, ventricose, free, watery white then pink. *Stem* short, straight or curved, smoky white or bluish grey, striately fibrillose, base not or very slightly swollen. *Basidia* four-spored. *Spores* pinkish, broadly elliptical, smooth with granular contents $7-8 \times 5-6 \mu$. *Cystidia* bladder-shaped of varied dimensions $25-40 \times 8-20 \mu$. *Cuticle* of pileus hymeniform with subglobose cells.

Habitat: on the ground in frondose woods. Collected by Mr E. A. Ellis at Wheatfen, 1 October 1942.

We follow Lange in naming this small agaric, which was described by Gillet from a sketch made by Godey. It is closely related to *Pluteus semibulbosus* which is characterized by the powdery surface of the pileus and very large cystidia.

Flammula flava (Bres.) Lange, *Fl. Ag. Dan.* iv, 12, Pl. 123, fig. G (1939).

Naucoria flava Bresadola, *Ann. Myc.* iii (1905) and *Icon. Myc.* tab. 795.

Flammula dactylidicola J. E. Lange in *Dansk Bot. Ark.* v, no. 7, 6 (1928).

Pileus usually small, full range 1-5 cm., fleshy, convex then gibbous or umbonate, margin incurved then straight, pale ochre when young turning to a deeper rust colour, the dry surface slightly tomentose. *Gills* crowded, pale lemon yellow then rusty, broad, arcuate or ventricose, emarginate with decurrent tooth, edge pallid and minutely denticulate under a lens. *Stem* 2-5 cm. long, 3-10 mm. diam. solid or with hollow base, fibrillosely striate, apex pruinose, pale yellow becoming rusty, slightly swollen below with fusiform base. *Flesh* soft, pale yellow. *Smell and taste* not specially noticeable. *Basidia* with four sterigmata. *Spores* ferruginous in mass, broadly ovate, indistinctly apiculate, minutely verrucose and sometimes one-guttulate, $4.5-5.5 \times 3.5-3.75 \mu$. *Cystidia* on gill edge in crowded groups, filiform capitate with base slightly swollen $25-30 \times 4-5 \mu$.

Habitat: in tufts of cock's-foot grass (*Dactylis glomerata*), two or three together but not caespitose. Collected by Mr G. J. Cooke at Lakenham Hall, Norwich, 29 August 1942, in an old overgrown orchard under a medlar tree.

This species is said to be not uncommon in Denmark but has been overlooked in this country probably because it is hidden at the base of grass stems. Lange usually found it attached to cock's-foot grass 'even in a mixed vegetation of more than ten species of grass, it was found exclusively on the *Dactylis* tufts'.

Galera appendiculata Lange & Kühner in *Le Genre Galera*, R. Kühner, 146 (1935) and *Fl. Ag. Dan.* J. E. Lange, iv, 36, Pl. 159, fig. A (1939).

Pileus 1-1.5 cm. convex then almost flat, umbonate or papillate,

ochraceous then pale buff, pulverulent, striate half-way or at margin, fugacious white veil appendiculate to margin in fragments. *Gills* crowded, adnate or almost free, ventricose or linear, cream at first, edge white and fimbriate. *Stem* 4-4.5 cm. long, 1.5-2 mm. thick, striate with white silky fibrils, pale honey below, pruinose above, slightly swollen at apex, base slightly bulbous covered with white pubescence. *Flesh* honey colour. *Basidia* four-spored. *Spores* in mass antique brown (Ridgway), elliptical, smooth with granular content $7-8 \times 4.5-5 \mu$. *Cystidia* abundant on gill edge, bottle-shaped with obtuse apex $25-45 \mu$ long. *Cuticle* of cap hymeniform with cells $12-20 \mu$ wide.

Habitat: waysides on naked soil or amongst grass in shady places. Found under hazel at Pewley Hill, Guildford, Surrey, 7 October 1942.

Not common but may be overlooked as the pendant marginal veil soon disappears, when it is not so easy to determine.

Galera ravida Fr. also has a marginal veil, but appears to be a more robust fleshy species.

G. appendiculata belongs to the section *Pholiotina* which includes species having a hymeniform cuticle of the pileus and no capitate skittle-shaped cystidia. In this section Kühner also includes several small species of *Pholiota*, but this is rather confusing.

Cortinarius (Inoloma) rubicundulus (Rea) n.comb., *Flammula rubicundula* Rea, *Grev.* xxii, 40, Pl. 185, fig. 2 (1894). *Cortinarius (Inoloma) pseudobolaris* R. Maire in *Cort. d'Eur.* (Bataille, 1912) and *Ann. Myc.* xi, 346 (1913). *Cortinarius (Telam.) limonium* Quél. non Fr. *Fl. Myc.* 139 (1888).

Pileus 4-8 cm. convex then expanded, margin incurved, aspect glabrous but *sub lente* with small innate scales, yellowish turning saffron red when touched; margin often showing remnants of veil which at first is white, then reddens. *Gills* fairly crowded, rather thick, linear or arcuate, adnate or with a decurrent tooth, often separating from stem, light ochre then cinnamon; edge smooth, at first pallid then saffron red. *Stem* 3-8 cm. long, 0.5-1 cm. thick, solid then hollow, cylindrical or with subbulbous base or sometimes with attenuated base, fibrillose striate, sometimes with minute granules forming a net at the apex. The white veil often forms a clear-cut zone or ring above this. *Flesh* cream, then yellowish, finally saffron. *Smell* faint. *Taste* mild or slightly bitter and acrid after mastication. *Spores* in mass milky coffee (antique brown: Ridgway), boat-shaped or subfusiform, very minutely punctate, 1-2 guttulate, $7-10 \times 4-4.5 \mu$. *Cystidia* on gill edge sparse fusiform $45-65 \times 7-9 \mu$.

Habitat: on the ground in deciduous woods. Rare.

Good illustrations of this species are in *Bull. Soc. Myc. Fr.* xxxix, Pl. VII, together with *C. bolaris* Fr. with which doubtless it is sometimes confused.

Cortinarius (Hydrocybe) holophaeus Lange in *Dansk Bot. Ark.* viii, no. 7 (1935) and *Fl. Ag. Dan.* iii, 45, Pl. 102, fig. C (1938).

Pileus 5-6.5 cm. convex, flat or with shallow umbo, hygrophanous, dark reddish brown often with black blotches or zones, deep buff when dry, covered with white veil when young which persists at margin in older

specimens. *Gills* distant, rather broad, adnate, ventricose, emarginate, edge smooth. *Stem* 4–7 cm. long, 1 cm. wide, striate with pallid silky fibrils, cartilaginous outside but with soft substance inside which soon rots; base swollen, sometimes oblique and covered with a white pubescence. *Flesh* brown. *Smell* and *taste* not distinct. *Basidia* four-spored. *Spores* in mass between amber and Sudan brown (Ridgway), ellipsoid sub-amygdaliform with granular contents, $8\text{--}9 \times 5\text{--}5.5 \mu$ smooth. The spores vary from elliptical to more distinctly almond-shaped.

Habitat: in beech woods. Found in Singleton Forest, Sussex, 9 October 1943.

Said to be rather rare, but probably one of the species we are apt to dismiss as *C. brunneus* which is sometimes found with the ring not clearly defined and has been variously interpreted.

Cortinarius (Telamonia) ammophilus n.sp. Pl. IX B.

Pileus 7–20 mm. conical then plane with persistent acute or obtuse umbo; fleshy; snuff brown umbo darker; rimose, sometimes breaking up into scales; margin regular, brown veil usually adhering to margin. *Gills* distant in three lengths, broad, ventricose, deeply emarginate, sometimes with anastomosing veins, edge blunt and smooth, somewhat wavy; light cinnamon at first. *Stem* 1–2.5 cm. long, slender or thick 1–3 mm. solid with a loose fibrous stuffing then hollow; at first with striate fibrils then brown; peronate; ring brown usually present, median or two-thirds up. *Flesh* white in pileus, with purplish tint in stem. *Smell* not distinct. *Taste* mild. *Spores* brown, elliptical, smooth, $8.5\text{--}11 \times 4.5\text{--}5.5 \mu$. *Cystidia* absent.

Habitat: in flat damp parts of sand dunes under *Salix repens*. Braunton Burrows, north Devon. October and November 1945, in troops. Collected by Dr F. R. Elliston Wright.

Differs from small conical species in the *Telamonia* group by its rimose cap, distant gills and purplish flesh. From the large quantities gathered, it may not be rare in sand dunes.

Type specimens: at the Herbarium, Royal Botanic Gardens, Kew. The figures on Pl. IX are by Dr R. W. G. Dennis. *Cortinarius* does not travel well and the specimens were not in their pristine colouring.

LATIN DIAGNOSIS: *Cortinarius (Telamonia) ammophilus* Pearson. *Pileus* carnosulus, umbrinus, rimosus vel interdum squamulosus e conico explanatus 7–20 cm. latus, umbone obscuriore, acuto vel obtuso, margine regulari, saepe cortina umbrina ornato. *Lamellae* valde distantes, latae, ventricose, postice eximie emarginatae, saepe venosae, cinnamomeo-ferrugineae, acie obtusa vel levì, aliquantulum undata. *Stipes* 1–2.5 cm. longus, tenuis vel crassus 1–3 mm., laxe farctus, albus vel brunneus, fibrillis sericeis striatus, peronatus, annulo medio vel supero persistente. *Caro* pilei alba, stipitis dilute purpureo tincta, odore subnullo, sapore dulciculo. *Sporae* ochraceae ellipsoideae leves $8.5\text{--}11 \times 4.5\text{--}5.5 \mu$. *Cystidia* nulla.

Habitat: catervatim, locis humidis, in terra arenosa prope mare.

Inocybe (Astrosporina) striata Bres. *Icon. Myc.* Pl. 759 (1930).

Pileus 7–10 cm. convex-plane with prominent pointed umbo, fleshy in centre, ridged at incurved margin, rimose then shaggy with adpressed or

upturned scales, almost smooth in centre, umber brown, almost black on umbo, paler towards margin. *Gills* subdistant, short ones near margin, somewhat narrow linear, adnate then free, whitish at first then colour of spores; edge white, fimbriate often spotted brown wavy. *Stem* 6–8 cm. long, 1–2 cm. thick, flabby with loose fibrous stuffing or hollow, striate with fibrils then shaggy, umber brown darker near base which is equal or slightly swollen. *Flesh* white in cap, pallid in centre of stem, brown near surface. *Smell* none. *Taste* rather rancid. *Spores* in mass, dilute snuff or tawny olive, nodulose, oblong $7-8 \times 5-6 \mu$ with five to six prominent nodules in outline. The spores are lighter in colour than in most species of *Inocybe*. *Cystidia* crowded on gill edge, sparse on face, fusoid ventricose with or without crested apex, $60-80 \times 14-18 \mu$.

Habitat: on sandy bank, near pine, birch and oak. Hindhead, Surrey, 23 September 1942.

Differs from Bresadola's diagnosis in larger size and somewhat smaller spores but differences would hardly justify a new specific designation.

Inocybe Langei Heim in *Le Genre Inocybe*, 335, Pl. IX, fig. 1 (1931). J. Lange, *Fl. Ag. Dan.* III, 78, Pl. 113, fig. F (1938).

Pileus 1–2.5 cm. convex then expanded usually with shallow umbo, pale buff or with slight pink tint, margin white, darker with age, rimose fibrillose, incurved margin shaggy and cracking. *Gills* subdistant, ventricose, rather broad, adnexed narrowly emarginate, white at first, then ochraceous with olive tinge, edge white and minutely fimbriate. *Stem* usually short, 2–4 cm. long, 4–6 mm. thick, white or flushed with pale pink, striate, pruinose above, solid, sometimes with bulbous base. *Flesh* white or with pink flush. *Smell* of new meal. *Spores* in mass snuff brown, elliptical, flat one side or almond-shaped smooth $7-8 \times 4.5-5 \mu$. *Cystidia* mostly short and broadly ventricose, apex blunt without neck or a very short one, usually crested $36-50 \times 14-17 \mu$.

Habitat: on bare ground. Growing in troops under laurel hedge. Hindhead, Surrey, 2 April 1942.

This small stubby *Inocybe* belongs to the series that may be described as satellites of *I. tomentosa* (Jungh.) Quél. better known as *I. eutheles* B. & Br. all of which are pale buff strongly smelling of new meal. The separate identity of these is not easy to determine, and one has largely to decide on the size and shape of spores and cystidia. *I. Langei* has short stout blunt cystidia. The spores vary in their outline, both ends being either round or pointed.

The small *Inocybe* described and figured by Bulliard as *Agaricus lanuginosus* has erect hairs on the pileus and its habitat is on or around dead stumps. Another and larger *Inocybe* is also commonly assigned to this species, but is distinct in many details. The smaller species should retain the epithet *lanuginosa*.

Inocybe (Astrosporina) lanuginosa (Bull) Fr., *Syst. Myc.* (1821) = *Inocybe ovato-cystis* Kühner & Boursier in *Bull. Soc. Myc. Fr.* XLIV, 181 (1928).

Pileus small 1–2 cm. wide, convex then flat, dark snuff brown, densely fibrillose with squarrose pointed scales in centre. *Gills* crowded, ventricose,

cream then brown with white fimbriate edge. *Stem* slender 3-4 cm. long, 2.5-3 mm. wide, cylindrical or flexuose, slightly swollen below, covered with rather shaggy fibrils, brown above, blackish brown below. *Flesh* white, turning brown at base of stem. *Smell* earthy. *Taste* mild slightly rancid. *Spore powder* snuff brown. *Spores* nodulose, with rough ovate or oblong outline, numerous conical warts and rather larger apical wart, mostly $8-8.5 \times 5-5.5 \mu$. *Cystidia* in tufts, ovate or pyriform with a few subcapitate ones, mostly without neck or crystalloid apex $35-40 \times 14-20 \mu$.

Habitat: on the ground. Hindhead, Surrey, round stump, September 1942. Henley, Sussex, under larch, October 1942, etc.

Not so common as the next. Cooke's figures in *Ill.* 408 (582) are more like *Inocybe dulcamara*, which doubtless is often confused with this species. His 409 (425) more nearly represents the above.

Inocybe (Astrosporina) longicystis Atk. G. F. Atkinson, *Am. J. of B.* v, 213 (1918).

Kühner & Boursier, *Bull. Soc. Myc. Fr.* XLIV, 179 (1928). R. Heim,

Le Genre Inocybe, 364 (1931) as *I. lanuginosa* forms *a* and *b*.

Pileus 3-4 cm. shaggy fibrillose with erect acute scales on disk, dark brown. *Gills* crowded, adnate, rather wide, pallid then snuff brown, with white fimbriate edge. *Stem* 4-7 cm. long, 5-6 mm. diam., cylindrical, brown with shaggy fibrils, pallid at apex, darker near base. *Flesh* white. *Smell* unpleasant. *Spore powder* snuff brown. *Spores* with oblong outline and many projecting warts, often with larger one at apex $8-10 \times 5-6 \mu$. *Cystidia* abundant, cylindrical or slightly ventricose and subcapitate, usually without crystalloid apex, but crystals are sometimes present $60-70 \times 15-17 \mu$.

Habitat: on the ground especially in damp woods. Fairly common. Grayswood, Surrey among sphagnum, September 1943. Rothimurchus Forest, Scotland, August 1938, and other localities.

This is fully worthy of specific rank. It is much larger in size and the cystidia are very different from those in *I. lanuginosa*. The spores are much the same as in preceding species except that the nodules are more numerous. This is only noticeable when the spores are examined together.

Inocybe serotina Peck. *Bull. N.Y. St. Mus.* 75, 17 (1904).

Pileus 1-8 cm. convex, obtusely umbonate, margin abruptly inturned at first, rimose with striate arachnoid fibrils, white turning ochre or rusty brown, splitting when old. *Gills* crowded, adnexed or free, ventricose or linear, white at first then snuff brown with a slight olive tinge and a white fimbriate rather thick edge. *Stem* 3-10 cm. long, 3-10 mm. wide, firm, solid, thickened above and below, base sometimes with a submarginate bulb, white near apex and with striate rusty fibrils below or rusty brown all over except at white base, often curved. *Flesh* white. *Smell* none or musty. *Taste* mild. *Spore powder* snuff brown. *Spores* cylindrical, smooth, $12-16 \times 5-6 \mu$. *Cystidia* usually not crested, closely packed on sterile gill edge, subcylindrical, balloon-shaped or ventricose $40-70 \times 10-14 \mu$; also on gill face.

Habitat: in sand dunes. Braunton Burrows, North Devon. Sent by Dr F. R. Elliston Wright, October and November 1945.

This North American species has already been recorded in the sand dunes of Holland and Denmark. It can readily be known by its oblong spores, the only other British species of *Inocybe* with cylindrical spores being the common *I. lacera*. It is very variable in size; the first gathering in October consisted of small specimens 1-2 cm. wide, but in November a robust example was collected with cap 6.5 cm. across and stem 10 cm. long.

Inocybe griseo-lilacina Lange in *Dansk Bot. Ark.* II, no. 7, 32 (1917) and in *Fl. Ag. Dan.* III, 73, Pl. 111, fig. F.

Pileus 1.5-2.5 cm., conical then flat usually with umbo but sometimes depressed in centre, margin involute at first, then straight, azure blue or lavender, disk turning brown, tomentose breaking into shaggy scales. *Gills* crowded or subdistant, white then snuff-coloured, ventricose, adnate, edge white, fimbriate. *Stem* 4-7 cm. long, 2.5-3 mm. thick, azure or lavender, densely covered with whitish flocci and fibrils, hollow but firm, cylindrical sometimes slightly swollen at base. *Flesh* azure blue or white in cap when old. *Smell* fairly strong, a blend of new meal and pelargonium. *Taste* mild. *Gill edge* sterile with thick-walled flask-shaped cystidia very varied in size and outline, with stout or slender neck, smooth or cristate apex; these are mixed with thin-walled sack-shaped cells (paracystidia) 12-20 μ wide. *Spore powder* snuff brown. *Spores* elliptical or sub-amygdali-form 8-9 \times 5 μ .

Habitat: solitary, in troops or caespitose, in deciduous woods, especially on roadside where leaves have been swept. Fairly common in south of England.

It is helpful to have this defined as a species separate from the less distinguished *I. obscura* (Pers.) Fr. to which it is allied. We take *I. obscura* to be the very common and smaller agaric with a bluish tint only at the apex of the stem.

The fig. C on Pl. IX is by Miss E. M. Wakefield from specimens collected near Haslemere, Surrey.

Stropharia rugoso-annulata Farlow, *Ic. Farl.* Pl. 65 (1929), also L. O. Overholts, *Mycologia*, XXII, 243, Pl. 30 (1930).

Pileus 5-12 cm. wide, fleshy, conical subglobose or flat, margin strongly incurved at first, livid purple (near vinaceous brown of Ridgway) with yellow background, innately fibrillose and rimose, sometimes pitted with holes on disk, covered with a thin pallid pruinous which disappears with rubbing; margin undulate and plicate. *Gills* up to 10 mm. broad, crowded, arcuate or broadly linear, adnate, smoky grey with white crenulate edge. *Stem* 15-17 cm. long, 1.5-3 cm. wide, solid but with loose fibrous stuffing, often narrowed at apex and abruptly broadening out to a long cylindrical stem with or without sub-bulbous base; white, washed with lemon; striate-ribbed; white tomentum at base, to which are attached white terete rhizomorphs. *Ring* superior, thick, white, tinted lemon frequently appendiculate to margin, the lower surface dividing up into large triangular segments, the upper surface fin-like, dovetailing between the gills, to

which it often remains persistently attached. When the ring is freed from the margin and the gills, it is pendulous, deeply sulcate above, smooth below with triangular segments at margin. *Flesh* white, unchangeable. *Gill edge* sterile with closely packed *cystidia* $12-16\mu$ wide, mostly clavate, sometimes broadly flask-shaped. *Spore powder* sooty black. *Spores* violaceous under microscope in water when fresh, the germ spore giving a somewhat truncate apex, broadly elliptical $11-13 \times 6-7\mu$.

Habitat: growing luxuriantly on rotting wheat straw. Woking, Surrey, September 1945. Brought to the Haslemere Museum by Miss V. Haslop. The seed was not of American origin.

Some etiolated specimens were also present, presumably having matured under the straw and looking remarkably different from the normal ones, the cap being quite flat, the colour almost white, the stem long and slender and not narrowed at the apex.

None of our specimens shed spores freely and only a sparse spore deposit could be obtained. The same experience seems to have been met with in America.

This striking species has only been recorded in recent years in the United States where it now appears to be fairly common. Its habitat there is on cultivated ground or among woody debris. The spores are variable. Farlow $11-13 \times 8-9\mu$; Overholts $13-15 \times 8-9\mu$; Rolf Singer (*in litt.*) $12-18 \times 7.5-10\mu$ mostly $13 \times 7.5-8\mu$. These are rather larger than my measurements.

The fine illustrations of *S. rugoso-annulata* on Pl. IX are by Dr R. W. G. Dennis and represent a typical specimen with the veil clinging to the gills. This does not bring out the triangular fringe of the veil which is better shown in the painting by Kroegeer in the *Icones Farlowianae*.

The photographs on Pl. X are by Major Maxwell Knight, and were taken *in situ*. Fig. A shows an etiolated discoloured specimen, which looks entirely different from the type.

Psalliota vaporaria (Vitt.) J. Schaeffer, *Ann. Myc.* xxxvi, 71 (1938). Vittadini, *Fung. Mang.* tab. viii (1835) as *Agaricus campestris* Linn. var. *pratensis*, *vaporarius*. J. Lange in *Fl. Ag. Dan.* iv, 59, Pl. 140, fig. D as *P. subperonata* Lange (1939).

Pileus 3-20 cm. fleshy, subglobose, margin inturned, dark brown, like a potato skin or darker (clay colour to tawny olive of Ridgway), background white covered with long ragged innate brown scales, dense in centre, less so at margin where the white flesh is evident. *Gills* crowded, dull pink when young then blackish brown, sublinear pointed both ends, free, edge pallid, fimbriate. *Stem* stout 14 cm. long, 4 cm. thick in specimens studied, white above, brown near base, cylindrical or slightly swollen below with a few shaggy scales. *Ring* double, the lower part sometimes separating from the upper, the top one white, striate clinging to apex of stem, the lower one very thick and partly brown, sometimes with a clear space between. *Flesh* white turning reddish brown. *Smell* slight of mushroom, but very variable. *Taste* mild, nutty. *Gill edge* sterile with small clavate or capitate cells. *Spores* blackish brown, subglobose $6-7 \times 5-6\mu$ mostly one-guttulate.

Habitat: in rich dark earth under trees by roadside. Grayswood, 28 August 1945. Collected by Dr F. Rauter.

No full description was given by Vittadini, only short explanations of the excellent figures on his plate. He clearly shows the separation of the two sections of the ring, which together with the almost globose spores, the ragged yet innately scaly pileus and the remarkably thick lower portion of the ring, make this a clearly defined species.

J. Schaeffer gives a choice of odours, from pine sawdust when fresh, to meat-gravy, liquorice and fenugreek (*Maggi*) when dry!

Psalliota xanthoderma Geneviev. *Soc. Bot. de Fr.* (1876).

This species is characterized by a silky, white, smooth, truncate pileus and marginate bulbous base to the stem. It turns a fugacious lemon yellow when bruised and has white flesh except in the base of stem which is yellow. The spores are very small and the gill edge sterile with subglobose cells. It is said to have an unpleasant 'foetid' smell which has variously been interpreted as creosote, carbolic acid, ink, the urine of mice, chlorine, phenol, iodoform, etc. The smell seems to be absent in most British specimens which have come under my observation, but has been noticed by some collectors. The conditions under which this smell occurs remain to be investigated. The type has been illustrated by Konrad and Maublanc in their *Icon. Sel. Fung.* Pl. 30, but the spore measurements given are much too large and they have not indicated the sterile cells on the gill edge which is an important diagnostic feature in many mushrooms.

There are many varieties and var. *lepiotoides* Maire has already been recorded by Rea. This differs from the type in having greyish brown concentric squamules on the cap. It is found in large rings on open chalk downs, the sporophores being sometimes of massive size.

Other varieties occur in Britain, and the following is fairly common, having been collected by me in Kent, Sussex, Surrey and Yorkshire.

Psalliota xanthoderma Genev. var. *obscurata* Maire in *Bull. Soc. Myc. Fr.* xxvi, 192, also as *P. meleagris* J. Schaeffer in *Zeitschrift für Pilzkunde*, Jahrg. 4, 28 (1925).

Differs from the type in having blackish squamules, dense in the centre and more scattered towards margin. The ring usually has flocci on lower surface. It is a very elegant mushroom with its black and white ('guinea-fowl') cap and grows under deciduous trees, especially oak, on the edge of woods where there is an accumulation of leaves or road sweepings. In the *Icon. Myc.* tab. 830 Bresadola has erroneously illustrated this variety as *P. silvatica* Schaeff. The spores taken from many gatherings measure $5.5 \times 3.5 \mu$ and agree with the spore deposit I obtained from a specimen named by Dr René Maire in Catalonia, Spain, in October 1935.

Another variety quite distinct from the above is var. *grisea* n.v. which differs from the type in the pale grey mat surface of the cap, which soon cracks and finally breaks up into minute squamules of the same colour. It has a ring with floccose fragments on the under surface and no smell. The spores are about $5 \times 4 \mu$ one-guttulate.

Habitat: on chalk grassland which had been trampled over by soldiers billeted nearby; near beech woods. Goodwood, Sussex, October 1942, November 1944.

LATIN DIAGNOSIS: *Psalliota xanthoderma* Genev. var. *grisea* Pearson. *Differt pilei superficie impolito, colore griseolo, deinde in squamulis minutis griseolis diffuso, odore subnullo. Sporae late ovatae vel subglobosae leves* $5\text{--}5\cdot5 \times 4\text{--}4\cdot5 \mu$ *uni-guttulatae. Cellulae aciei lamellarum obovatae.*

This mushroom lacks the elegance of the other varieties and may deserve specific rank, but a more intensive study of the whole group is called for.

Psalliota villatica Brond. sensu Bres.

Pileus 5–30 cm. very fleshy, at first subconical or globose with inturned margin, then expanding to semi-globose, mat pulverulent breaking up into small or large adpressed concentric scales, white or pale buff, yellow when rubbed, very variable in colour when old, in dry sunny weather turning a golden yellow like ripe wheat, often with reddish patches, veil often hanging in ragged patches to margin. *Gills* crowded, broad or narrow; linear, ventricose or arcuate, pointed both ends, free, edge smooth, at first pallid grey, then a pinkish brown, finally blackish brown, mostly of one length many forked from stem. *Stem* robust 5–20 cm. long, 3–5 cm. thick, firm, solid, white, cylindrical or ventricose, with concentric white or brown flocci below ring when young, striate above, smooth and satiny when old when it may have a slight salmon tinge. *Veil* peronate two-thirds up, the loose part or ring smooth above with a few floccose squamules below, wide or narrow; large part of veil often adheres to margin of pileus. *Flesh* white turning salmon pink in stem, sometimes only in patches. *Smell* variable, in young stages of anise or almonds, but often most unpleasant when old. *Taste* mild, slightly rancid when raw, but a very good edible. *Spore powder* brownish black. *Spores* elongate pip-shaped $10\text{--}12 \times 5\text{--}6 \mu$. *Gill edge* sterile with club-shaped cells $8\text{--}10 \mu$ wide; sometimes there are globose or ovate cells in clusters, but it is uncertain whether this is merely an adventitious feature of no diagnostic importance.

Habitat: in pastures and especially abundant on chalk soil. Collected in Surrey, Sussex and Hampshire but doubtless present everywhere.

This rather puzzling mushroom, which may vary with weather and soil, is usually determined as *P. arvensis*, from which it differs in the larger spores and coloration, especially the salmon-pink colour of the flesh when exposed to the air.

It is most difficult to come to a conclusion as to its correct name, but we have taken the line of least resistance by adopting the epithet used by Bresadola (*Icon. Myc.* tab. 829) and Lange (*Fl. Ag. Dan.* iv, 56, Pl. 139) though it cannot be assigned with certainty to the original *villatica* of Brondeau in *Mem. Linn. Par.* v, lxvi (1828). However, in view of its variability the Brondeau species may well be the same, for he accentuates the squamulose covering of the stem '*marqué d'écailles très saillantes dans la jeunesse*'.

Miss E. M. Wakefield, who has paid much attention in recent years to

the genus *Psalliota*, associates her name with mine as joint author of the following two species:

***Psalliota arenicola* n.sp.**

Pileus 2-7 cm. wide, fleshy, very compact and firm, semi-globate then flat or slightly depressed in centre, smooth, silky, white, persistently involute, cuticle overlapping the gills and shaggy. *Gills* very crowded, free, at first very pale pink, finally brownish black, ventricose or wedge-shaped, edge white and fimbriate. *Stem* 3-4 cm. long, 1-1.5 cm. thick, cylindrical, equal or slightly swollen at base, solid and hard, white, turning reddish when rubbed, the lower part peronate to middle at first, soon breaking up into ring-like fugacious fragments, smooth and silky when old, a distinct ring not always present and when present very narrow with shaggy edge. Long mycelial threads are sometimes at the base of the stem. *Flesh* white turning brownish red in lower part of stem. *Smell* not distinctive. *Taste* mild. *Spores* in mass, blackish brown; subglobose $5-6 \times 4.5 \mu$, one-guttulate. *Basidia* four-spored. *Gill edge* sterile with sack-shaped cells about 10μ wide.

Habitat: in sand, often so buried that the top of cap only is showing; in troops. Braunton Burrows, North Devon, November and December 1944, November 1945. Sent by Dr F. R. Elliston Wright.

Type specimens at the Herbarium, Royal Botanic Gardens, Kew, Surrey.

LATIN DIAGNOSIS: *Psalliota arenicola* Wakefield & Pearson. *Pileus* 2-7 cm. *latus*, *albidus*, *carnosus*, *solidus*, *e convexo planus vel sub-depressus*, *glabratus*, *sericeus*, *marginem semper involuto, cute lacerata ultra lamellas excedente*. *Lamellae confertissimae, liberae, e pallidis rubescentes, demum nigro-brunneae, ventricosae vel cuneatae, acie alba minute fimbriata*. *Stipes* 3-4 cm. *longus*, 1-1.5 cm. *crassus*, *cylindraceus*, *vel basi sub-bulbosus, solidus, firmus, albidus tactu rubescens, deorsum primo peronatus, deinde veli fragmentis annulatis ornatus demum glabratus sericeusque*. *Annulus angustus, mox evanescens*. *Caro alba ad basim stipitis rubescens, odore subnullo et sapore grato*. *Sporae nigro-brunneae late ellipsoideae vel sub-globosae* $5-6 \times 4.5 \mu$. *Basidia tetraspora*. *Acies lamellarum sterilis cellulis clavatis circa 10μ latis*.

Habitat: in terra arenaria prope mare, saepe in arena sepulta, summo pilei modo visibili.

***Psalliota litoralis* n.sp.**

Pileus 7-8 cm. wide, convex with flat or depressed centre, fleshy, light buff or biscuit colour, becoming darker and finally pale tawny brown or ochraceous tawny (Ridgway), fibrillose at first, breaking up into small or large flat scales, margin incurved exceeding gills and shaggy with appendiculate veil. *Gills* very crowded, free, rather wide, pointed both ends, at first pallid, finally black with slight purplish tinge; edge white, fimbriate. *Stem* 3-4 cm. long, 1.5-2 cm. thick, cylindrical, solid, white, striate above ring, reddish brown below. *Ring* median or two-thirds up, lower part sheathing stem, upper part spreading 8-10 mm. *Flesh* white in pileus, reddish brown in stem. *Smell* faint, not distinctive of mushrooms. *Spore powder* mummy brown (Ridgway). *Spores* elliptical apiculate $6-8 \times 4.5-5 \mu$, one-guttulate. *Gill edge* sterile with long cylindrical cells $5-6 \mu$ wide.

Habitat: in short turf exposed to sea breezes. Saunton Down, North Devon, June 1945. Sent by Dr F. R. Elliston Wright.

Type specimens at Herbarium, Royal Botanic Gardens, Kew, Surrey.

LATIN DIAGNOSIS: *Psalliota litoralis* Wakefield & Pearson. *Pileus* 7-8 cm. *latus*, *carinosus*, *ex hemisphaerico expansus vel depressus*, *alutaceus*, *dumum obscurior denique fulvo-brunneus*, *cuticula primo fibrillosa deinde in squamulas vel squamas adnatas diffissa*; *margo incurvatus ultra lamellas excedens*; *velum album ad marginem lacerato-appendiculatum*. *Lamellae confertissimae, liberae c. 5 mm. latae e pallidis purpureo-fuscae, acie albo-fimbriata*. *Stipes* 3-4 cm. *longus*, 1.5-2 cm. *diam. cylindraceus, solidus, supra annulum albus striatusque, subter rufo-brunneolus*. *Annulus membranaceus* 8-10 mm. *latus, in stipite medio, infra in vaginam productus*. *Caro pilei alba, stipitis rufo-brunnea, odore grato*. *Sporae nigro-brunneae ellipsoideae apiculatae* 6-8 × 4.5-5 μ *uni-guttulatae*. *Acies lamellarum sterilis cellulis cylindraceis* 5-6 *latis*.

Habitat: in caespite ad ventum marinum exposita.

Boletus cramesinus Secr. *Mycographie Suisse* (1833). *B. sanguineus* (With.)

Fr. var. *gentilis* Quél. (1883). *B. tenuipes* (Cooke) Massee in *Br. Fung.*

Fl. 1, 281 (1892).

Pileus 3-12 cm. *viscid, smooth or wrinkled, pinkish (crushed strawberry) speckled with a lighter pink*. *Tubes bright chrome or golden yellow, deeply channelled round stem, decurrent in fine lines down apex of stem*. *Pores angular, medium, concolorous, sometimes rufescent when bruised*. *Stem usually slender, sometimes more robust and ventricose, narrowed to a point at base, yellow above, brownish or rufous below*. *Flesh white with faint flush of pink, and a deeper pink near surface*. *Smell not distinctive*. *Taste mild*. *Spore powder clay colour*. *Spores fusiform* 12-13 × 4.5-5 μ. *Cystidia fusiform bright yellow mostly about* 60 × 16 μ (50-70 × 14-20 μ, Konrad & Maublanc).

Habitat: mixed wood at Shillinglee, Sussex, 25 October 1943, and at Ruislip Wood, Middlesex, 1 October 1944. Said by Gilbert to be often found on charcoal heaps.

B. cramesinus has nothing to do with *B. sanguineus* (With.) Fr. which is a doubtful species with flesh that turns blue. F. Kallenbach, in his unfinished monograph of *Boletus*, has a good series of figures under the name *B. auriporus* Peck, but from Peck's description it is far from certain that it is the same; anyway Secretan's epithet has priority. The fungus is also well illustrated in the *Ic. Sel. Fung.* of Konrad & Maublanc, Pl. 413 as *B. sanguineus* var. *gentilis*. Normally it is a small *Boletus*, but in September 1945 a specimen with a pileus 12 cm. wide was sent to the Herbarium, Royal Botanic Gardens, Kew, which differed from the type in the large size and in that the chrome yellow pores turned a blood red when bruised. Type specimens of *B. tenuipes* (Cke) Massee have been examined by Dr Dennis and he found brilliant yellow cystidia present, which is a clear indication that it is the same species as described above.

Boletus scaber is a binomial which represents to most European mycologists the common *Boletus* of birch woods with a grey or blackish pileus, minutely tomentose *sub lente*, a rough stem dotted with small scales

and flesh that remains unchangeable when exposed to the air or only has a very faint flush of pink.

It has, however, been claimed in France, and first, we believe, by the eminent mycologist René Maire, that the true *scaber* of Bulliard as figured on Pl. 132 (1809) is another and less common *Boletus* with flesh that turns black. It has been described and illustrated by F. Kallenbach as *Boletus pseudo-scaber* F. Kall. (1935), an epithet which is not valid, since it was used in 1833 by Secretan for another species.

There has been a lively discussion about the matter and the French point of view has been put forward in various papers in the *Bull. Soc. Myc. Fr.* I adopted the names used by the French authors when I prepared the *List of the Fungi of Epping Forest*, published by the Essex Field Club (1938), but on looking into the matter more closely the reasons put forward do not appear convincing. Bulliard's description and figures are not good, and no decision can be based on them. If a French tradition did exist as to the identity of Bulliard's species, it was kept in the background and came to light for the first time in 1933 when the *Bull. Soc. Myc. Fr.* included in its list of species the unfamiliar name of *Boletus leucophaeus* Pers. to indicate the common birch bolet, the one with the white unchangeable flesh, though in the original diagnosis of *B. leucophaeus* the flesh was described as blackening when exposed to the air, so even this name was inappropriate.

However, what almost amounts to a universal tradition cannot be cast aside in this light manner. All that was required was a new name for a 'new' fungus. The new *Boletus* certainly has some features that make it worthy of this distinction. A study of the literature discloses the fact that it had already been described in the 1924 edition of Michael's *Führer für Pilzfreunde* by R. Schulz as *B. scaber* var. *Carpini*, and when raised to specific rank it is to be hoped that this name will be accepted.

Boletus Carpinii (R. Schulz) Pearson. *B. scaber* Bull. var. *Carpini* R. Schulz, *Führ. f. Pilzfr.* (1924). *B. pseudo-scaber* Kall. non Secr. *Die Pilze Mitteleuropas*, 1, *Die Röhrlinge*, 117, Pl. 40 (1935). *B. scaber* Maire et al.

Pileus 4-6 cm. wide semi-globate, glabrous but usually strikingly wrinkled, often only in patches or with smooth centre and wrinkled towards margin, then cracked in a network of rather large meshes, pruinose when quite fresh, light buff, snuff brown, milky coffee or tawny olive (Ridgway). *Tubes* cream, very long in relation to cap and projecting beyond margin of pileus, free. *Pores* cream, dirty brown when rubbed. *Stem* 5-13 cm. long, usually slender but ranging from 10 to 40 mm. thick, deeply sulcate and ribbed, attenuated above, with small dark brown or black scales arranged longitudinally on a greyish white background. *Flesh* white at first, slowly turning first a violaceous pink then a mottled black. *Cuticle* of pileus with globose cells 10-12 μ diam. *Spores* in mass, snuff brown; fusiform apiculate 15-17 \times 4.75-5 μ slightly hooked one end near apicle.

Habitat: in deciduous woods and copses, especially under hornbeam (*Carpinus Betulus*) and hazel (*Corylus Avellana*); late summer and early

autumn. Found in Surrey, Sussex and North Devon early September 1943-5.

This species which has only recently been recognized as distinct from *Boletus scaber* is really very different. Once known we are surprised that it should have been missed. It has been confused with *Boletus duriusculus* which always grows under poplars, is more robust and cracks, but the cracks make a finer mesh and the surface of the pileus has a stippled appearance.

The above description of *Boletus Carpini* is from specimens gathered at Hindhead, Surrey. A coloured plate from a drawing made by Dr Dennis appears in the *Naturalist*, April 1946.

I have to thank Dr J. Ramsbottom for helping me to a decision as to which epithet should be applied to this much-discussed species.

How are we to cite the authors for *Boletus scaber*? In the *Systema Mycologicum* (1821) *B. scaber* is a collective species so the usual citation *B. scaber* (Bull.) Fr. has little meaning. Krombholz was, I think, the first to describe fully the species as we know it. It is true he did not mention the birch, but the habitat given is just where the birch tree appears: common on heaths, wood clearings and coppices, never in the depths of the forest. If Quélet (1888) is given as the doubtful authority for *scaber* in the French sense, Krombholz (1836) may be cited for the species with unchangeable flesh. *Boletus scaber* (Bull.) Krombh. will at least be unambiguous, but perhaps *B. scaber* auct. will suffice.

There is a variety of *B. scaber* which should be added to our British list. I have come across it two or three times and doubtless would have found it more often if I had taken the trouble to examine the flesh of every specimen of *B. scaber* met with. It was first pointed out by R. Singer in the Swiss journal *Schw. Zeitschrift für Pilzkunde*, xvi, 137 (1938) who then named it *Krombholzia scabra* var. *chromapes* (Frost) which was not acceptable as Frost's species is very different. In the *Ann. Myc.* xl, 32 (1942), he renamed it var. *coloripes* Sing. and under this name it should be recorded.

Boletus scaber var. *coloripes* Sing. Differs from the type in the flesh being golden or sulphur yellow at base of stem, turning a blood red or a greenish blue. First received from Capt. A. Dunston, Donhead-St-Mary, Wiltshire, 21 October 1941; also collected at Hindhead, Surrey, 23 September 1942.

These colour changes require further observation.

Hydnum (Dryodon) cirrhatum (Pers.) Fr. Pl. XI.

On 22 September 1943, not far from Goodwood, Sussex, we had the good fortune to find a magnificent specimen of this beautiful rare fungus, growing on a prostrate oak trunk. The series of sessile pilei were loosely and irregularly imbricate and as can be seen in the photograph, are of varying size. Further details are as follows: fleshy, rather fragile convex, flat or undulate, margin incurved at first, pale cream staining yellow when bruised or broken. *Top surface* covered with spines, erect in centre, prostrate towards margin. *Hymenium* with long spines 5-15 mm. long, subulate, cream, rusty pink when rubbed and when old. *Flesh* thick behind, thin at

margin, white, unchangeable. *Smell* pleasant. *Taste* mild. *Basidia* with 2 long sterigmata. *Spores* in mass Cambridge buff (Ridgway), amyloid, subglobose $3-3.5\mu$ often one-guttulate. *Spines of pileus* with masses of conidia similar to spores. *Cystidia* long filiform cells on hymenium which soon break down and are not visible when old.

Pl. XI is from a photograph by Mr Colin G. Fletcher and is about two-thirds the natural size.

Irpex pachyodon (Pers.) Quél. *Fl. Myc.* 377 (1888). *Sistotrema pachyodon* (Pers.) Fr.

Pileus 3-14 cm. wide, sessile, imbricate with 1-5 cm. thick shallow ridges 1-2 cm. apart; surface even, pubescent, white then cream (light buff of Ridgway). *Hymenium* of stalactite spines and plates, pointed or dentate, of varying lengths and widths, colour of pileus. *Flesh* white, soft at first then coriaceous. *Spores* in mass, pale buff; subglobose with 1 large guttule $5-6\mu$ in our specimens ($5-8 \times 4.5-6.5\mu$, Bourdot & Galzin). *Cystidia* none. *Hyphae* $2-4\mu$ diam. usually without clamp connections; a few may be present, see Bourdot & Galzin.

Habitat: on bark of living holm oak. Near East Dean, Sussex, September-November 1943-5.

Pl. XII is from a photograph kindly taken by Mr F. Ballard.

Clavaria versatilis Quél. *Ass. Fr.* (1893).

Receptacle 6-12 cm. high, violaceous drab, then brownish drab with darker tips, finally powdery with the pale ochraceous spores; trunk naked, hollow, white at base, violet above and drab at top, divided into thick cylindrical or compressed branches which are not closely compact but rather loose and not pressed against each other; more or less dichotomously divided into numerous branchlets with toothed apices which may be violet when fresh but are usually ferruginous brown. *Flesh* white unchangeable, inodorous. *Basidia* long and narrow $30-40 \times 6-8\mu$. *Spore powder* pale ochre (chamois of Ridgway). *Spores* oblong apiculate (sub-fusiform) with granular contents $8-9 \times 4.5-5\mu$ minutely aculeate. *Hyphae* not clearly defined, of various diameters.

Habitat: on bank under beech trees. Henley Hill, Sussex, September and October 1942-3. Quélet's epithet has been used, but *C. fennica* Karst. would have priority if it is the same species. It is certainly very close, but its habitat is in coniferous wood. *C. versatilis* is said to be not uncommon in the beech woods of France.

Clavaria botrytis (Pers.) Fr. var. *alba* n.v.

All parts pure white except the spores which are the normal colour, pallid ochre (chamois of Ridgway). Several tufts were growing on a bank under beeches at Henley, Sussex, October 1942.

(Differt a typo colore omnino albo; sporis dilute ochraceis ut in typo.)

EXPLANATION OF PLATES IX-XII

PLATE IX

Fig. A. *Stropharia rugoso-annulata* Farlow.

Fig. B. *Cortinarius ammophilus* Pearson.

Fig. C. *Inocybe griseo-lilacina* Lange.

Fig. D. *Mycena arcangeliana* Bres. var. *Oortiana* Kühner.

PLATE X

Stropharia rugoso-annulata Farlow.

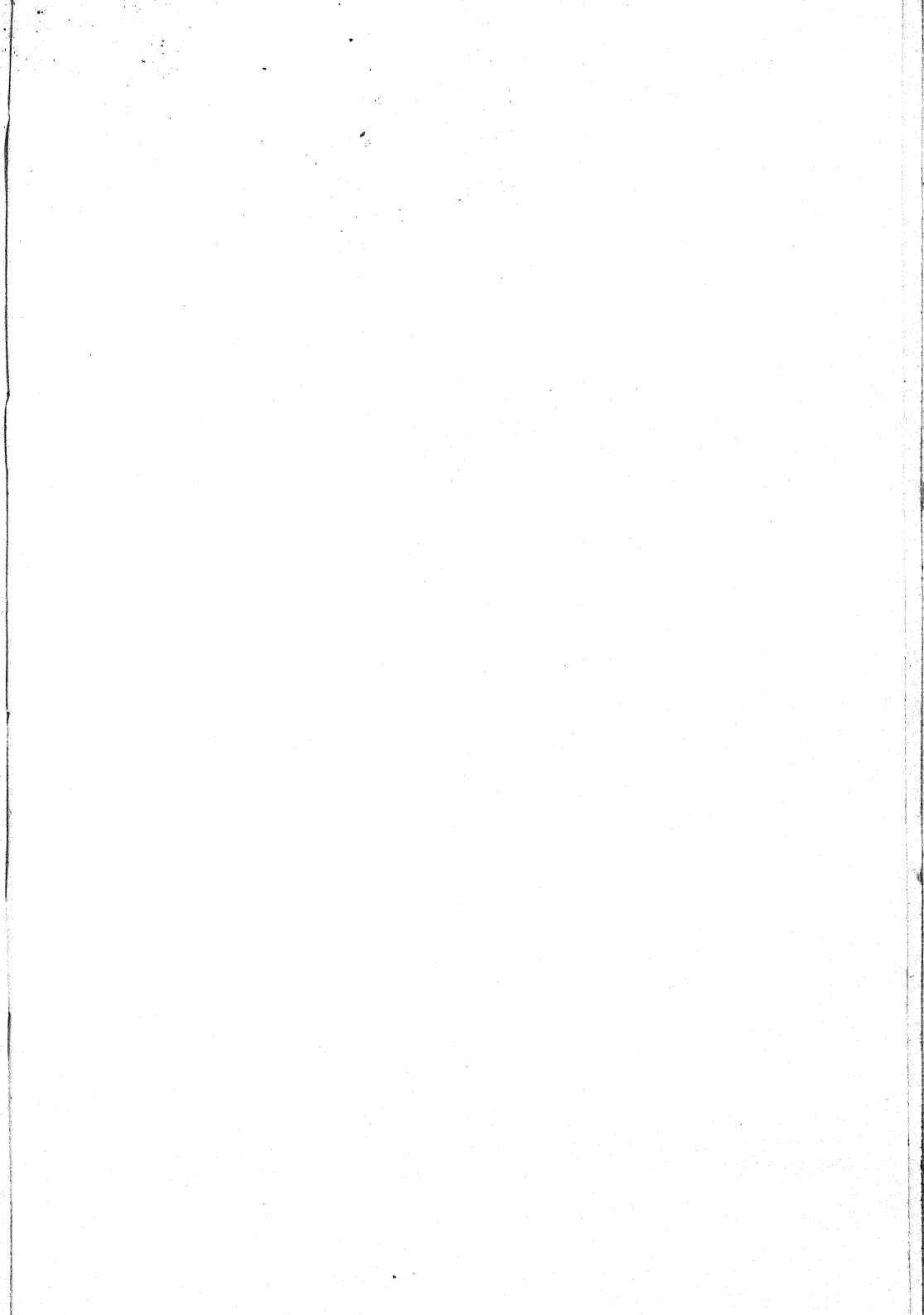
PLATE XI

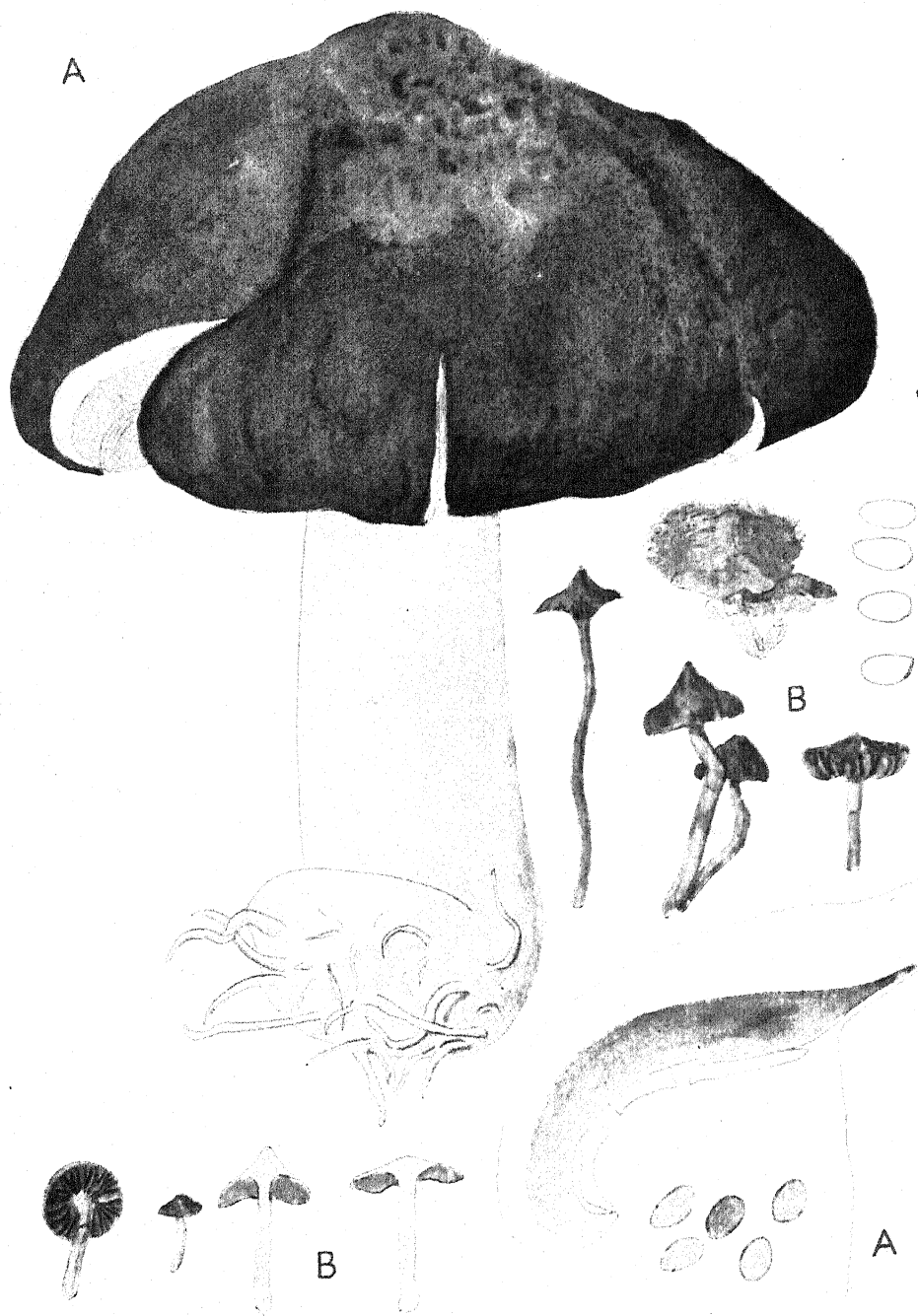
Hydnum cirrhatum (Pers.) Fr.

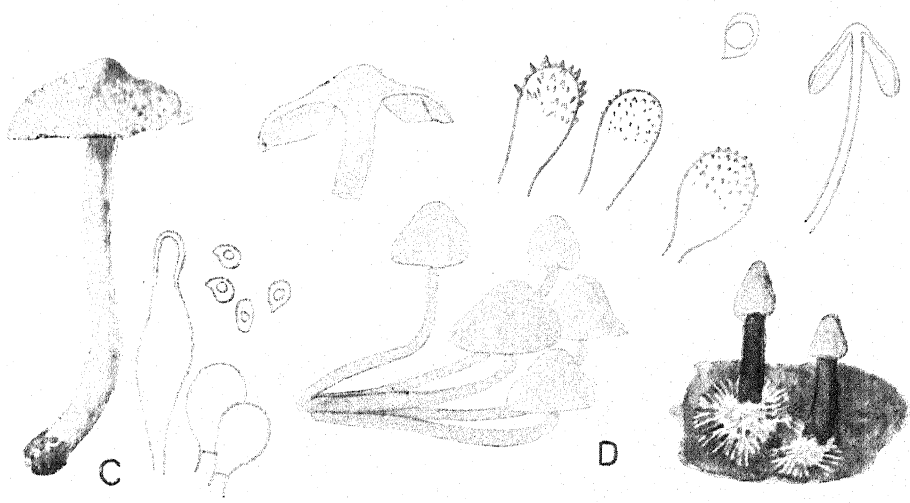
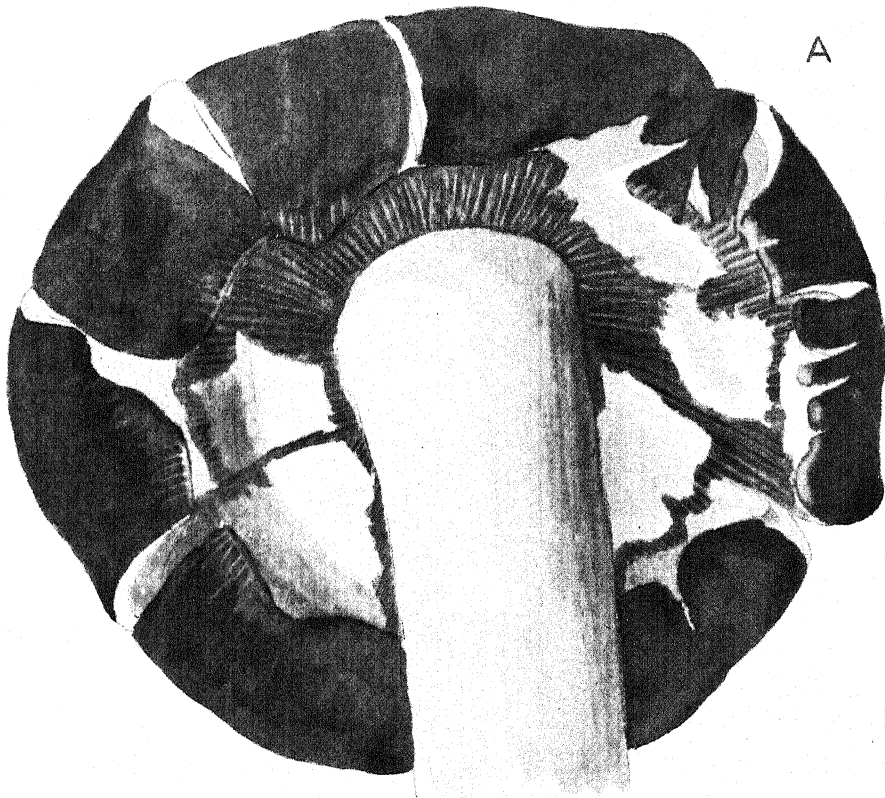
PLATE XII

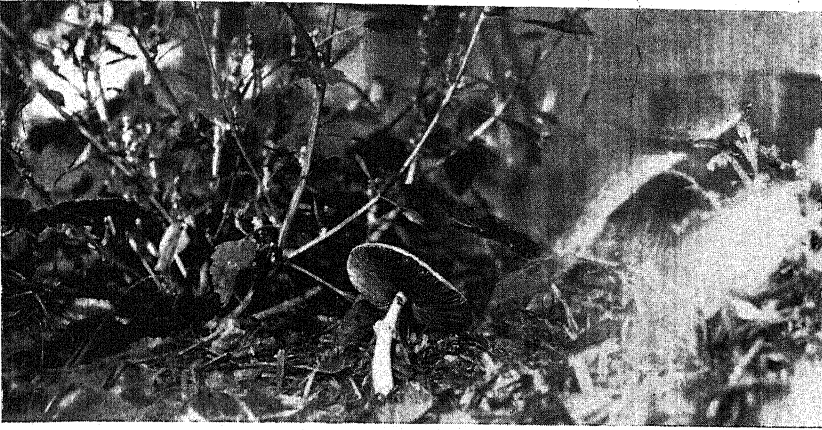
Irpex pachyodon (Pers.) Quél.

(Accepted for publication 18 March 1946)







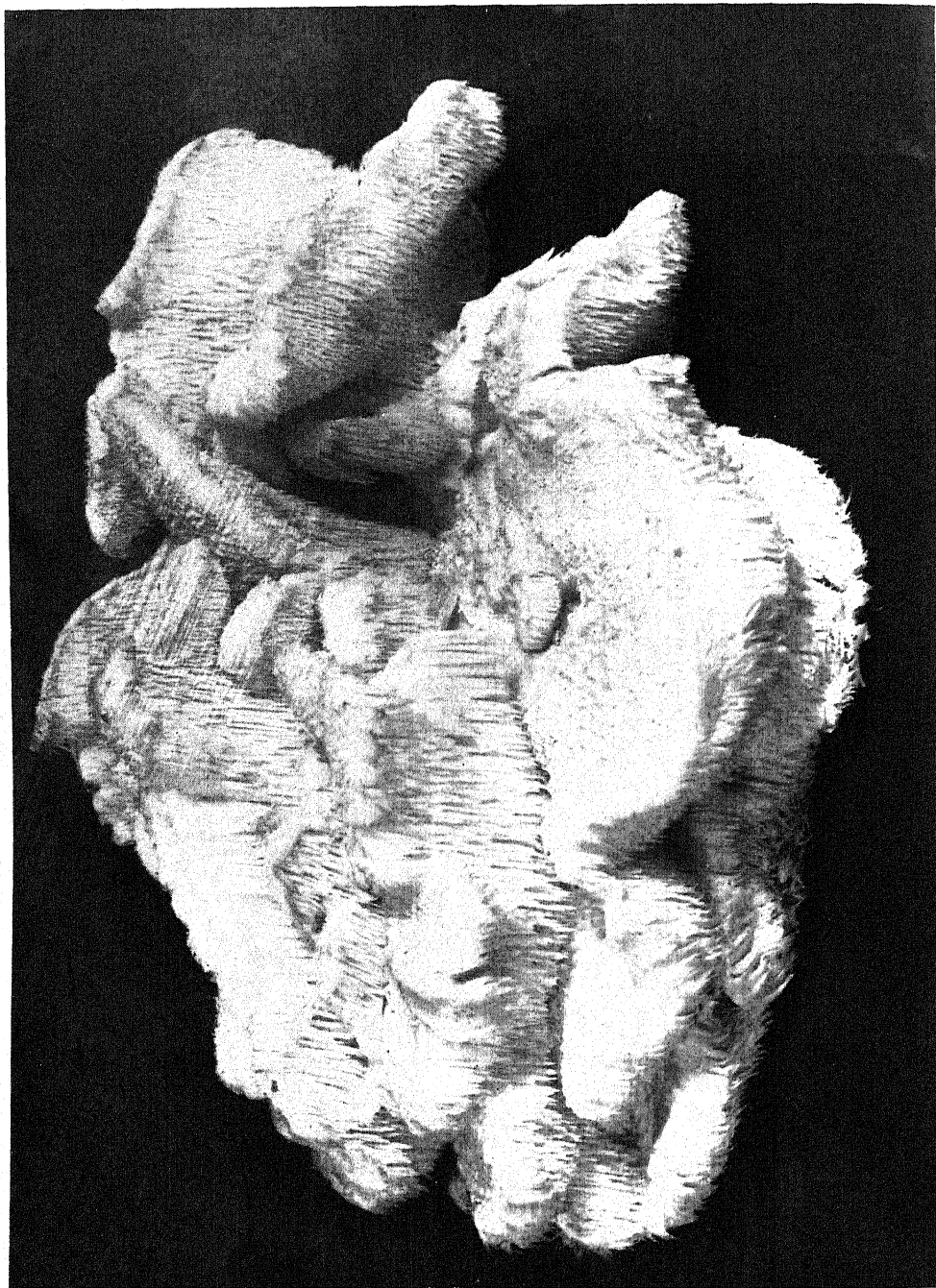


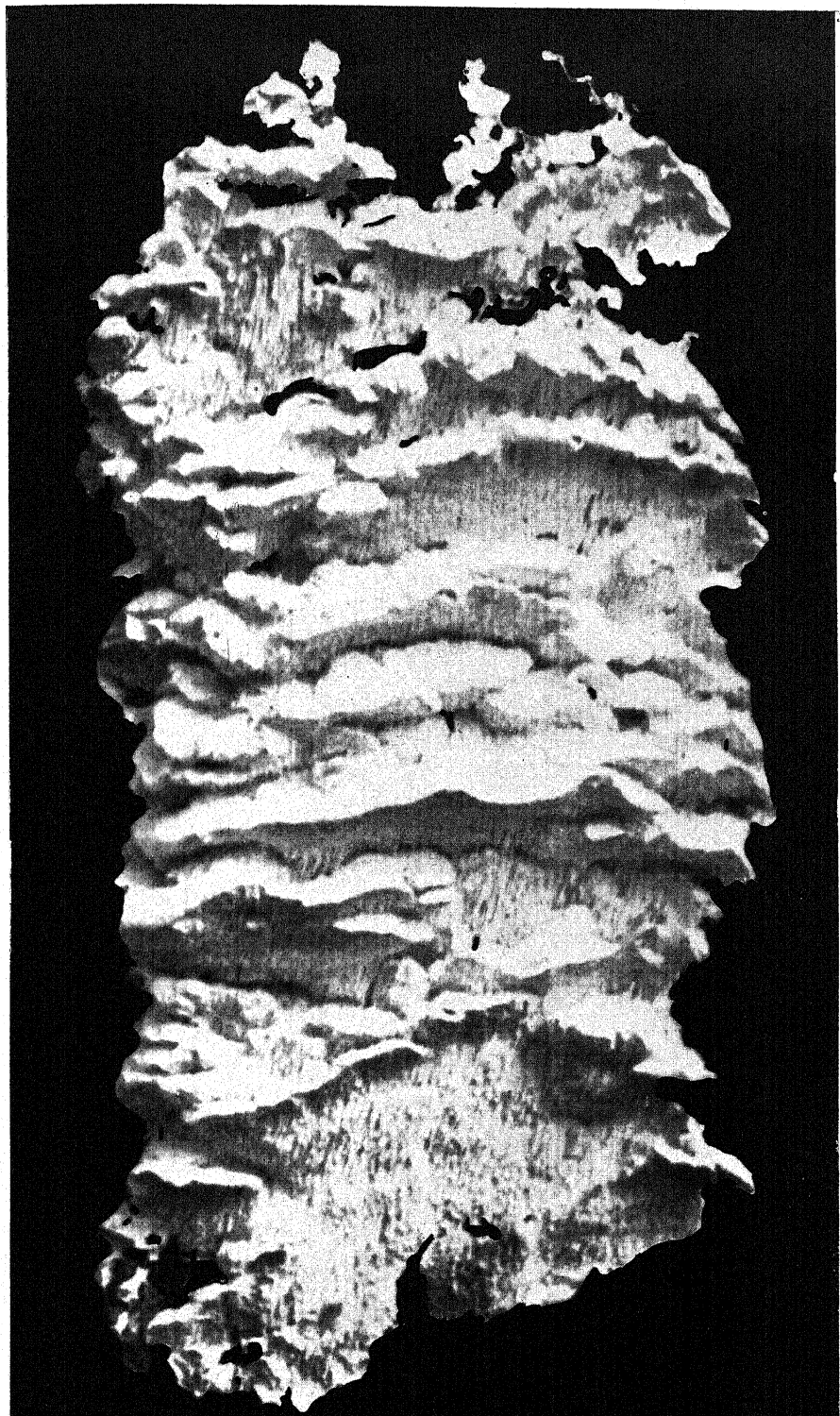
A



B







PRODUCTION OF GLIOTOXIN BY *PENICILLIUM TERLIKOWSKII* ZAL.

By P. W. BRIAN, *Hawthorndale Laboratories, Jealott's Hill Research Station*

Gliotoxin was one of the first antibiotic substances to be isolated in pure form (Weindling & Emerson, 1936) and has since been found to be a metabolic product of a variety of moulds. A list of those fungi which have been said to produce gliotoxin is given below:

Aspergillus fumigatus Fres. (Menzel, Wintersteiner & Hoogerheide, 1944).

Aspergillus fumigatus mut. *helvola* Yuill (Glistler & Williams, 1944).

Penicillium Jansenii Zal. (Brian, Hemming & McGowan, 1945).

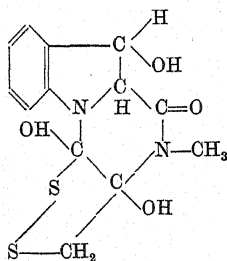
Penicillium obscurum Biourge (Mull, Townby & Scholz, 1945).

Penicillium sp. (unidentified) (Johnson, McCrone & Bruce, 1944).

Trichoderma viride Pers. ex Fries (Weindling & Emerson, 1936; Brian, 1944).

Whether gliotoxin is produced by *Gliocladium fimbriatum* Gilman & Abbott or not, is open to some doubt; it seems probable that the organism used under that name by Weindling (1937) was, in fact, a strain of *Trichoderma viride* (Brian, 1944). The organism described above as *Penicillium Jansenii* is now identified as *P. Terlikowskii* Zal.

The chemistry of gliotoxin has been intensively investigated (Johnson, Bruce & Dutcher, 1943; Johnson *et al.* 1944; Bruce, Dutcher, Johnson & Miller, 1944; Dutcher, Johnson & Bruce, 1944; Johnson, Hasbrouck, Dutcher & Bruce, 1945; Dutcher, Johnson & Bruce, 1945), the most probable structural formula for gliotoxin, in the opinion of these workers, being



The biological properties of gliotoxin have also been widely studied. It is actively antibacterial (Waksman & Woodruff, 1942; Johnson *et al.* 1943; Rake, Jones & McKee, 1943; Schatz & Waksman, 1944; Brian & Hemming, 1945), Gram-positive and Gram-negative bacteria being equally sensitive; of interest is the recent discovery of its great *in vitro* toxicity to *Mycobacterium tuberculosis* (Schatz & Waksman, 1944). It is highly fungistatic; saprophytic and plant pathogenic fungi are sensitive (Weindling,

1941; Waksman & Bugie, 1943; Brian & Hemming, 1945; Brian *et al.* 1945; Reilly, Schatz & Waksman, 1945) and also a number of dermatophytic fungi (Reilly *et al.* 1945; Sanders, 1946). Gliotoxin is toxic to mammals (Johnson *et al.* 1943).

In view of the considerable amount of information available concerning gliotoxin, this communication is confined to a brief description of the conditions under which it is produced by *Penicillium Terlikowskii* with a discussion of its natural importance.

SOURCE AND CHARACTERISTICS OF *PENICILLIUM TERLIKOWSKII*

The strains of *P. Terlikowskii* used in this study were isolated from Wareham Heath soil and were originally identified as *P. Jensenii* (Brian *et al.* 1945). Wareham Heath soil is directly or indirectly toxic to coniferous trees (Rayner, 1934). This toxicity, associated with a general low level of microbiological activity, was shown to be of biological origin by Neilson Jones (1941); it has since been suggested (Brian *et al.* 1945) that production of gliotoxin by the organism now identified as *P. Terlikowskii*, which is very abundant in this soil, may be one factor involved.

Colonies of our isolates on Czapek-Dox agar are closely felted, almost velvety. The colony showed no folding and the centre is only slightly raised; the margin is white, 3-4 mm. in width. Conidial areas are sage green and the reverse initially white, slowly developing traces of pink. Colourless transpiration drops are formed, some becoming yellowish or reddish in the older parts of the colony. There is no odour. The penicilli are monoverticillate, borne on short branches from a fertile hypha. The sterigmata are 8.5-10 μ in length, bearing conidial chains in compact columns. The conidia are smooth and globose, 2.5 μ in diameter.

This is fairly close to the description of *P. Jensenii* Zal. given by Thom (1930), the main points of difference being the absence of any complex penicilli and the absence of obvious connectives on the conidia. Two strains (241 and 244) were submitted to Dr K. B. Raper (United States Department of Agriculture) who has kindly commented as follows: 'Your strains 241 and 244 are tentatively identified as *P. Terlikowskii* Zaleski. These strains are monoverticillate and do not agree with Zaleski's figures which place *P. Jensenii* in the Lanata-Divaricata of Thom. If we interpret the strains as belonging in the Monoverticillata-Ramigena, your strains do key to *P. Jensenii*. If they are interpreted as Stricta-Funiculosa with ropiness reduced, the strains key to *P. Terlikowskii* and agree with the species description fairly well except conidiophores appear smooth and not rough, as described by Thom.'

As these strains do appear to agree more closely with *P. Terlikowskii* than with *P. Jensenii*, although there is still an element of doubt in the diagnosis, they are referred to throughout this paper under the former name.

This mould shows the dual phenomenon described by Hansen (1938) very strongly. Cultures made by mass conidial transfer exhibit a continuous tendency to produce white mycelial overgrowths, which swamp the conidial areas. Transfers of this mycelium remain sterile. It is shown

later in this paper that the mycelial form is much less antibiotically active than the conidial form.

EXPERIMENTAL METHODS

In all the experiments described here the procedure has been to set up replicate cultures on 30 ml. lots of medium in 100 ml. Pyrex flasks, five such cultures being withdrawn at random and bulked for assay of fungistatic activity at appropriate intervals. The composition of the Weindling, Raulin-Thom and Czapek-Dox media has been previously given (Brian, Curtis & Hemming, 1946). All cultures were incubated at 25° C.

Assays of fungistatic activity have been carried out by a serial dilution spore germination test, using conidia of *Botrytis Allii* Munn. The method has been described in detail by Brian and Hemming (1945).

Table 1. *Development of fungistatic activity (in B.A. units/ml.) in cultures of Penicillium Terlikowskii on four standard media*

Strain no.	Days incubation	Medium			
		Weindling	Czapek-Dox	Raulin-Thom	Cornsteep
241	6	32	16	12	16
	8	96	24	64	24
	10	48	24	48	16
	12	64	24	32	8
245	6	8	8	16	16
	8	8	8	16	32
	10	12	8	8	12
	12	8	8	8	8
244	6	2	—	8	4
	8	—	—	4	4
	10	—	2	12	—
	12	2	4	8	2

RELATION OF COMPOSITION OF MEDIUM TO DEVELOPMENT OF FUNGISTATIC ACTIVITY

Comparison of standard media

Table 1 shows the development of fungistatic activity in culture filtrates from three strains of *P. Jensenii* growing on four standard media. It will be seen that of the three strains no. 241 is the best; all experiments subsequently described refer to this isolate or various substrains derived from it. Of the four media, Weindling and Raulin-Thom were best, though the superiority of these two media over Czapek-Dox or Cornsteep was not so marked as is the case with gliotoxin production by *Trichoderma viride* (Brian & Hemming, 1945).

Variation of carbon source in Weindling medium

Using isolate no. 241 on a Weindling medium with various pure carbon sources, it was found that maltose, lactose, galactose and dextrin were most suitable for development of fungistatic activity, followed closely by starch, dextrose and sucrose; no activity developed in media containing carbon as ethyl alcohol, glycerol, mannitol, citrate or tartrate.

Concentrations of dextrose from 1 to 5% have been tested. Increase from 1 to 2% increases the final activity, but further increase in dextrose concentration was not of advantage.

Variation of nitrogen source in Weindling medium

In similar experiments to those just described it was found that sodium nitrate, ammonium tartrate, ammonium sulphate, ammonium nitrate and peptone were equally suitable nitrogen sources.

Initial pH of Weindling medium

Weindling medium was adjusted to various pH values, ranging from 2.3 to 6.0, with hydrochloric acid or sodium hydroxide. The development of fungistatic activity and the drift of pH in culture filtrates, after growth of *Penicillium Terlikowskii*, are shown in Table 2.

Table 2. *Development of fungistatic activity (B.A. units/ml.) in cultures of Penicillium Terlikowskii on Weindling medium adjusted to various initial pH values*

Initial pH of medium	Fungistatic activity				pH			
	6	8	10	12 days	6	8	10	12 days
2.3	8	8	8	8	1.9	1.2	2.1	2.0
3.0	16	8	8	8	2.2	2.2	2.5	2.2
4.4	32	32	48	48	2.9	2.8	3.2	2.9
5.2	32	48	48	32	3.1	2.9	3.4	3.1
6.0	32	64	48	48	3.1	3.1	3.6	3.6

Growth was good on all the media, but sporulation was poor on the two with lowest initial pH (2.3 and 3.0). The media with high initial pH were best for development of fungistatic activity. It is well established that gliotoxin rapidly decomposes at reactions of pH 4.0 or higher, and it is therefore of interest to note that the pH of all media rapidly fell to pH 3.1 or less.

Accessory factors

In the experiments detailed in Tables 1 and 2 an impure form of dextrose (crude glucose chips) was used in the media. In the course of other experiments it was noted that this crude glucose was much superior, both for growth of *P. Terlikowskii* and for development of fungistatic activity, to pure dextrose. Spectrographic examination of the two samples showed the impure form to be higher in iron, manganese and zinc. The effect of addition of minor elements to media containing pure dextrose or crude glucose was therefore examined. Minor elements were added at two levels, the lower level being based on the minimal requirements for *Aspergillus niger* as determined by Steinberg (1935). The minor elements added were:

Element	Concentration ($\mu\text{g./ml.}$)	
	Low level	High level
Fe	0.2	1.0
Cu	0.03	0.15
Zn	0.2	1.0
Mn	0.02	0.1
Mo	0.02	0.1

The results of such an experiment are shown in Table 3. It is clear that addition of the minor elements did not bring the pure dextrose media up to the level of those containing crude glucose though sporulation was slightly improved.

Similarly, it was found that addition of calcium salts, also present in the crude glucose, did not increase fungistatic activity in pure dextrose media though again sporulation was slightly improved.

Table 3. *Effect of minor elements on development of fungistatic activity (B.A. units/ml.) in cultures of Penicillium Terlikowskii on Weindling media containing pure dextrose or crude glucose*

Grade of dextrose	Days growth	Minor element level		
		None	Low	High
Pure	6	4	—	2
	8	4	—	4
	10	8	2	—
Crude	6	12	12	12
	8	24	64	16
	10	32	32	24

Organic supplements such as peptone, yeast extract, aneurin hydrochloride, nicotinic acid, riboflavin, inositol, β -indolyl acetic acid or pyridoxin similarly had no effect on the development of fungistatic activity. The nature of the stimulatory material in crude glucose therefore remains unknown.

STRAIN VARIATION

It has been mentioned earlier that *P. Terlikowskii* shows a tendency to produce a sterile white mycelial form which outgrows the conidial form. In a number of experiments very low fungistatic activity of culture filtrates was associated with predominance of the mycelial form. From strain no. 241 it was found possible by single-spore cultures to isolate sterile mycelial forms, intermediate forms showing sparse sporulation and conidial forms sporulating more or less vigorously. These latter single-spore cultures still showed the tendency to produce the sterile mycelial form in sub-cultures. Several of these single-spore strains were compared on Weindling medium for production of fungistatic activity; results are shown in Table 4. These results show quite clearly that the purely mycelial forms are valueless for gliotoxin production, the conidial forms being outstandingly superior.

THE NATURAL SIGNIFICANCE OF GLIOTOXIN PRODUCTION

Gliotoxin has been shown to be produced by *Aspergillus fumigatus*, *Trichoderma viride*, *Penicillium obscurum* and *P. Terlikowskii*, and in all probability will be found to be produced by yet more mould species. Of these, *Trichoderma viride* and *Aspergillus fumigatus* are widespread and abundant in soil, *Trichoderma viride* in all parts of the world, *Aspergillus fumigatus* more particularly in the warmer regions. *Penicillium Terlikowskii* has been shown to be abundant, at least in certain soils, in England, and was first recorded by Zaleski (1927) from a pine-forest soil in Poland.

Consideration of other antibiotic substances confirms the existence of a strong correlation between production of antibiotic substances by micro-organisms and the soil habitat. The mould antibiotics penicillin and claviformin (also known as clavacin and patulin) are each known to be produced by several distinct fungi, in the main characteristically found in soil. The actinomycete antibiotics streptomycin, streptothricin, actinomycin, actinomycetin, proactinomycin and others are produced by soil actinomycetes. Most of the bacteria known to produce antibiotic substances are typical soil organisms.

Most antibiotic substances of microbial origin, then, are characteristically produced by soil organisms, and, in the present state of our knowledge of the distribution of the organisms concerned, it would appear that those producing gliotoxin are particularly widespread.

Table 4. *Comparison of single-spore strains of Penicillium Terlikowskii for production of fungistatic culture filtrates*

Strain no.	Type	Activity (B.A. units/ml.)		
		6	8	10 days
241/1	Mycelial	—	—	—
241/2	Mycelial	6	4	4
241/3	Intermediate	12	12	6
241/4	Conidial	128	64	32
241/5	Conidial	64	48	32
241/6	Conidial	64	32	24
241/7	Conidial	128	64	64

The role of antibiotics in nature has not yet received the study which the subject deserves. It is easy to appreciate that, in a closed environment such as the soil, the secretion into the surrounding medium of a substance toxic to other organisms utilizing similar nutrients may be of considerable competitive value, and hence of evolutionary significance, to the organism producing the antibiotic. On the other hand, if this were the case one would expect that antibiotic-producing strains of soil fungi would be favoured by natural selection and would become the dominant strains. Experience has shown that, on the contrary, strains producing high yields of antibiotics are relatively uncommon, and strains of the same species producing none at all are quite common. These facts illustrate the need for biological studies in relation to antibiotic-producing soil micro-organisms. It would be of interest to study, for instance, the survival of conidial gliotoxin-producing strains of *P. Terlikowskii* and the mycelial strains of low antibiotic activity; in pure culture the mycelial strains are most vigorous, but in soil the situation might be very different. The distribution of viridin-producing, gliotoxin-producing and antibiotically inactive strains of *Trichoderma viride* also seems worthy of investigation, and similarly with strains of other micro-organisms producing antibiotic substances.

The production of antibiotics probably profoundly affects the maintenance of microbiological equilibria in the soil. The toxicity of Wareham Heath soil to certain mycorrhizal fungi has been attributed by Brian *et al.* (1945) to accumulation of gliotoxin. Support for this view has been

obtained by correlation of the sensitivity to gliotoxin of various mycorrhizal and other soil fungi with their observed abundance and distribution on Wareham Heath (Rayner, 1945). Newman and Norman (1943) have suggested that the low level of microbiological activity in subsurface soils is due to leaching down of antibiotic substances produced by micro-organisms in the surface soil. It seems reasonable to suppose that many other aspects of microbiological balance, such as the phenomenon of soil sickness and its cure by partial sterilization, which may have a considerable effect on soil fertility, are at least in part concerned with the production of antibiotic substances by soil micro-organisms. In the more acid soils, where it would be relatively stable, gliotoxin is probably of great importance in this connexion.

SUMMARY

A *Penicillium* producing gliotoxin, isolated from Wareham Heath soil, has been identified as *P. Terlikowskii* Zal.

Culture filtrates from this mould developed greater fungistatic activity on Raulin-Thom and Weindling media than on Czapek-Dox or a Corn-steep medium. In Weindling, a wide variety of carbon sources may be used and ammonium, peptone and nitrate nitrogen is suitable as a nitrogen source. The initial pH of the medium should be above pH 4.0.

An impurity in a crude grade of glucose used in some of the culture media was found to increase sporulation and to stimulate greatly development of fungistatic activity in culture filtrates. It has not been found possible to associate this effect with various minor elements (iron, copper, zinc, manganese, molybdenum), calcium, various vitamins (aneurin, nicotinic acid, riboflavin, inositol, pyridoxin, β -indolyl acetic acid), peptone or yeast extract.

There is considerable variation in the capacity to produce gliotoxin among strains of *P. Terlikowskii*; a common sterile mycelial variant, though vigorous in growth, produces little, if any, gliotoxin.

The possible significance of gliotoxin as a factor concerned in the maintenance of microbiological equilibria in soil is discussed.

I am indebted to Miss Myrtle Bray and Mr H. G. Hemming for much technical assistance during the course of this investigation.

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THE OCCURRENCE OF PERITHECIA OF THE OAK MILDEW IN BRITAIN

By NOEL ROBERTSON AND IAN MACFARLANE

During a foray to Bricketwood, Hertfordshire, on 6 October 1945, six perithecia (cleistocarps) of the Oak Mildew were found on a living leaf of *Quercus Robur* L. Many living leaves bearing the mildew were examined, some from the same tree and some from other trees nearby, but no more perithecia were found. This collection, like those of other powdery mildews hitherto found on oaks, proved to belong to the genus *Microsphaera*, which is distinguished from the other genera of Erysiphaceae by the presence of more than one ascus in the perithecium and by the characteristic dichotomous branching of the tips of the appendages. The mycelium was abundantly developed, mainly on the adaxial surface of the leaf, and bore elliptical to barrel-shaped conidia measuring $25-37 \times 15-22 \mu$ with an average size of $31 \times 19 \mu$. The perithecia were $180-200 \mu$ in diameter, the appendages, twenty to twenty-four in number, were from 170 to 300μ in length and the spores (twenty from three perithecia) measured $18-24 \times 6-13 \mu$.

The perithecia of the Oak Mildew have been recorded from many European countries at infrequent intervals (Blumer, 1933), the earliest record being that of Arnaud and Foëx (1912) from south-west France in 1911. The perithecia have only recently been recorded from those countries with a maritime climate: they were found in Norway in 1932 (Jørstad, 1945) and in Holland in 1939 (Hartsuijker, 1939), and have still to be recorded from Sweden and Belgium. It is generally believed that the development of the perithecia is inhibited in a maritime climate, whereas their development is encouraged by periods of hot, dry weather. Laibach (1930), using potted plants, has shown that by keeping the soil dry, or by reducing the relative humidity of the surrounding atmosphere (or by combining both treatments) he was able to induce the production of perithecia. Conditions at Bricketwood seemed dry for the time of the year, and the number of terricolous Hymenomycetes was abnormally low according to those who had visited the wood in previous autumns. Weather records collected at Rothamsted (eight miles away) showed that the total rainfall in the thirty-three days from 4 September to 6 October 1945, was about half (1.113 in.) that of the average for the same period in the ten previous years (2.25 in.), but, in the absence of extensive data on the subject, we do not wish to imply any causal relationship between the dryness of the season and the occurrence of the perithecia.

We have identified the fungus provisionally with *Microsphaera alphitoides* Griff. & Maubl. (1912), since it agrees closely with the description of the original authors and also with that of Blumer (1933). We have matched our specimen with the exsiccatus no. 3099 of Sydow's *Mycotheca Germanica*, issued as *M. alphitoides*, and find that it agrees closely. The average diameter of the perithecia collected at Bricketwood is 180μ compared with Blumer's

report of 116μ (range $103\text{--}130\mu$), but the other characters agree, and we think that we are dealing with the same fungus. If this variation in different collections is caused by environmental conditions, it is possible that the perithecial diameter should not have the importance given it by Blumer in his delimitation of species. We have seen one collection of *M. densissima* (Schw.) Cooke & Peck (collection by Gerard from the vicinity of New York, in Herb. M. C. Cooke, 1885 in the herbarium of the Royal Botanic Garden, Edinburgh), from which it is difficult to differentiate *M. alphitoides* on morphological grounds alone, although the distal dichotomy of the appendages in *M. densissima* appears to be more elaborate. This last character is a variable one, however, and we have observed some perithecia in which the appendages were very similar to those of *M. alphitoides* (our own specimen and Sydow's no. 3099). It may be worth noting that the type collection of *M. alphitoides* Griff. & Maubl. is the perithecial material collected by Arnaud and Foëx and identified by them with the American species *M. quercina* (Schw.) Burr. Further study of the variation of the American Oak Mildews may show that the European form could be included as one variant within a complex. Since the first discovery of its perithecia in 1911 the descriptions and collections of the Oak Mildew in Europe have been consistent, the fungus is distinctive among the oak mildews in the massive development of a thick, white mycelium and, as far as is known (see Neger, 1915), it is more or less confined to the European oaks, with records on the beech (*Fagus sylvatica* L.) and the chestnut (*Castanea sativa* Mill.). This combination of uniformity with host range and geographical distribution suggests that it is useful to maintain *Microsphaera alphitoides* as the name of the European Oak Mildew, until further investigation of the variability and host range of the American oak mildews, which have been classified differently by almost everyone who has studied them, finally settles the question of its identity.

Permanent mounts of single perithecia have been made and deposited in the British Museum (Natural History), the Herbarium of the Royal Botanic Gardens, Kew, the Herbarium of the Royal Botanic Garden, Edinburgh and in the Cambridge Botany School.

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OBSERVATIONS ON WILT DISEASE OF FLAX

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(With Plate XIII and 2 Text-figures)

In 1942 an investigation was begun on the effects of certain seed treatments on the prevention of seed-borne diseases in oil varieties of flax. The fungicides used during the first year's trials were Nomersan and Agrosan G, applied dry in the form of a fine powder at the rate of 0.6 %, and the flax varieties tried were La Plata (a collection of regional strains from the Argentine) and Bison and Redwing (consisting of uniform, carefully selected seed from Canada).

The distribution of pathogenic fungi on the untreated seed was determined by the Ulster method of Muskett and Malone (1941) and is shown in Table 1. The percentage of seed infected with any one of these diseases was 1 % or less.

Table 1. *Distribution of pathogenic fungi on flax seed*

Flax variety	Pathogenic fungi
La Plata	<i>Botrytis cinerea</i> Fr.
Bison	<i>Colletotrichum linicola</i> Pethybr. & Laff.
Redwing	<i>Botrytis cinerea</i> Fr. <i>Fusarium Lini</i> Bolley = <i>F. oxysporum</i> Fr. f. <i>Lini</i> (Bolley) <i>Fusarium culmorum</i> (W. G. Sm.) Sacc. <i>Polyspora Lini</i> Laff.

The treated and untreated seeds were sown in randomized, replicated rows eight inches apart at a rate equivalent to fifty pounds per acre. The date of sowing was 20 May 1942, exceptionally late for flax.

The prolonged drought of April and May had broken with slight rain two days before sowing commenced, but the soil was still warm at the time of planting. The weather continued showery with bright intervals for the next fortnight. Mainly warm, dry weather followed for the rest of June. July, on the whole, was cool, dull and showery, but improved towards the end of the month when the plants were in flower or beginning to fruit. The ripening period during August was warm and damp.

Of the fungi carried on the seed which were potential sources of disease, two caused serious trouble in the field. *Polyspora Lini*, inconspicuous at the seedling and stem-break stages, became epidemic during the later browning phase when the warm, damp weather of August favoured its development. *Fusarium Lini* was even more important, especially during the early stages in the growth of the crop.

Before the end of the third week after sowing it was noticed that many seedlings were dying. The cotyledons were not marked by any lesions, but became dull and limp and the edges rolled up. At ground-level the base of the hypocotyl or the top of the radicle had a thin, constricted appearance. The diseased seedlings drooped over on to the ground and died. In hot,

sunny weather the dead seedlings dried up into fine, dry, brittle threads, but on still, warm, humid days they became covered with a white mycelium which could also be seen on small lumps of soil carried up on the testa, and even growing on the cotyledons and on the top of the hypocotyl of living seedlings. When brought indoors and incubated in a damp atmosphere at 25° C., the diseased seedlings invariably became covered with a mycelium which soon produced micro- and macroconidia and later chlamydospores subsequently identified as those of *F. Lini* (Wilson, 1944). The symptoms of this seedling disease agree with those of wilt disease caused by *F. Lini* described by Bolley (1901*a*) and Millikan (1945), and, less fully, by several other authors.

Seedlings of all varieties were affected to some extent, but particularly those of Redwing, the original source of the infection, and of La Plata. At later stages La Plata was so much more susceptible that the rows of this variety could be picked out from the rest by the large number of dying

Table 2. *Mortality due to wilt disease in 1942 crop*

Variety	Treatment	Seeds per ft. at 50 lb. per acre	Seedlings per ft.		Mature plants per ft.
			Alive	Dead	
La Plata	None	45	20	6	9
	0.6% Nomersan	45	24	4	8
	0.6% Agrosan G	45	20	10	8
Bison	None	58	34	4	22
	0.6% Nomersan	58	39	1	28
	0.6% Agrosan G	58	31	5	19
Redwing	None	68	35	8	21
	0.6% Nomersan	68	40	10	29
	0.6% Agrosan G	68	39	9	25

plants which they contained (Table 2). La Plata is known to be very susceptible to wilt. Redwing is generally regarded in North America & resistant and Bison as very resistant (Dillman, 1928, 1936; Allison as Christensen, 1938; Borlaug & Christensen, 1941; Schuster, 1944), but these two varieties, especially Redwing, are more susceptible to the strains of *F. Lini* in New Zealand and Australia (Bayliss, 1940; Millikan, 1945).

When older plants were attacked, the cotyledons and lower leaves of the plant wilted, turning yellow from the edge inwards and then becoming brown and withered and later falling off. The top of the plant sometimes drooped over to one side. Finally, the infection spread until the whole plant was withered and dead. Unlike the wilted seedlings, the dead plant remained erect. The base of the stem at soil level which at first was thin and wrinkled, owing to the partial destruction of the cortex, later became much thickened, a feature of the disease to which attention was drawn by Barker (1923). The roots were less noticeably affected than the shoots, but sometimes showed decorticated areas.

It will be noticed from Table 2 that the rate of spread of the fungus within the first twenty days was extremely rapid. Edwards (1945) has found that the diameter of the mycelium of the fungus on 2% malt agar adjusted

to pH 7.5 and kept at 23° C. (optimum conditions) is only 50–52 mm. after five days. It therefore seems impossible that the fungus could have grown through the soil from the infected seed of Redwing to attack plants in other rows eight inches away, in twenty days. Bolley (1901 *a, b*) states that conidia, which are formed in great numbers on the surface of the soil, may be carried long distances in surface drainage waters, and that small quantities of infected soil or pieces of sick plants blown by the wind or carried on the feet or on agricultural implements may be enough to start the disease in clean soil, but he does not consider that the wind dispersal of conidia occurs to any marked extent. It has been observed, however, that when steam-sterilized soil is artificially infected with a culture of *F. Lini* and kept at 23–24° C. in a humid atmosphere, microconidia are produced on the surface of the soil within twenty-four hours of inoculation and macroconidia appear soon afterwards. Uninoculated pots of steam-sterilized soil, placed at a distance of three feet from infected pots, invariably become diseased under these conditions, and this can only be due to the air dispersal of the conidia. Such warm, humid conditions are rarely found out of doors, but did occur during the early stages of germination in 1942 when the surface growth of the fungus on the soil and on dead seedlings was observed. No evidence of long-distance transmission of conidia was found.

Of those plants which survived in the 1942 crop, many of all varieties were conspicuously stunted. The stunted plants were more common in the varieties Bison and Redwing than in La Plata. Some were only a few inches high, many did not flower at all and others produced only a single capsule. Among the dwarfed plants and also in rows where large sections had been completely wiped out, there stood up a few conspicuously well-grown plants which had either enjoyed complete immunity or which the infection had not reached. Most of the few remaining plants of La Plata fell into this category.

Isolations of *F. Lini* were made, not only from the original seed of Redwing, but also, after surface sterilization, from seedlings, from the stem and root of older wilting plants, from stunted plants of all varieties and from the seed produced by the crop. These isolates were used to infect pots of steam-sterilized soil in which healthy seed of La Plata was sown. When kept at a temperature of 23–24° C. all the plants developed the symptoms of wilt disease noted in the experimental plots, and died within periods varying between twenty to thirty-seven days. It was possible to re-isolate the fungus from the dead seedlings. The fungus thus proved even more virulent under these conditions than in the field.

The distribution of diseases on the seed gathered from the 1942 crop was determined by the Ulster method and is shown in Table 3.

The seed produced from the untreated Redwing was the most heavily infected with *Polyspora Lini*, and was therefore selected for testing the efficacy of Nomersan and Agrosan G in controlling this fungus when the seeds were tested by the Ulster method. It was found in one test using 400 seeds that Nomersan reduced the infection of *P. Lini* from 55 to 2 %, while Agrosan G suppressed it altogether. For testing these fungicides against *Fusarium Lini*, seed from Bison was used as, although this was the most

resistant of the three varieties, its seed was the most heavily contaminated. Using the same method as before, it was found that the infection was reduced from 14 to 3 % with Nomersan and to 1 % with Agrosan G.

In 1943 an attempt was made to infect the soil in one bed in which *F. Lini* had been noted in the previous season more thoroughly with this fungus, and for this purpose infected seed from the 1942 crop was sown. This seed also carried *Polyspora Lini*. As might have been expected, the crop was a poor one and disease caused by both fungi was prevalent.

By 1944 the patches of soil infected with *Fusarium Lini* were fairly evenly distributed over the plot, and an attempt was made to find out whether the fungicides which had given a considerable measure of control in laboratory tests were equally effective in the field. Seed of La Plata (the

Table 3. *Distribution of seed-borne diseases on 1942 crop*

Variety	Treatment of seed before sowing	% of seed of crop infected with		
		<i>P. Lini</i>	<i>B. cinerea</i>	<i>F. Lini</i>
La Plata	None	21	0	1
	0.6 % Nomersan	9	1	4
	0.6 % Agrosan G	12	0	0
Bison	None	21	3	14
	0.6 % Nomersan	19	3	2
	0.6 % Agrosan G	6	3	3
Redwing	None	55	0	0
	0.6 % Nomersan	38	0	0
	0.6 % Agrosan G	38	0	0

most susceptible variety to wilt available), which was free from all seed-borne diseases except about 0.5 % of *Polyspora Lini*, was chosen. Some was treated with 0.6 % Nomersan, some with 0.6 % Agrosan G and some was left untreated. With these, three kinds of plots were sown in the infected soil on 7 May 1944. Each type of plot was triplicated.

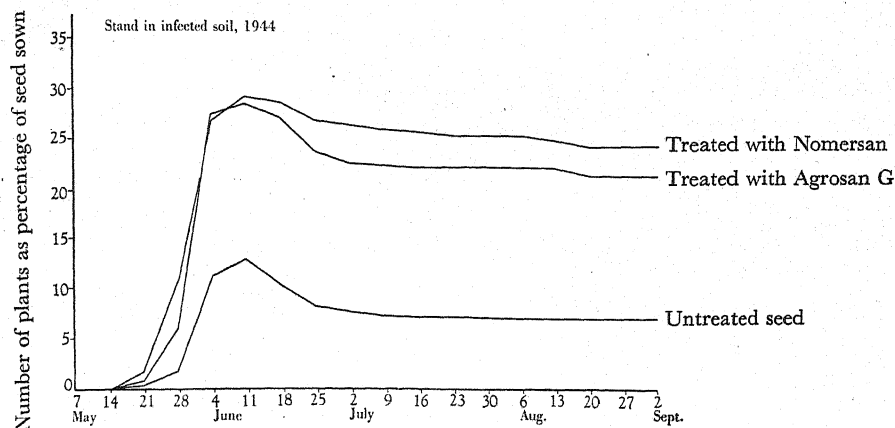
There was some disease caused by *P. Lini*, but this was not conspicuous until the browning phase when the crop was ripening. Wilt, due to *Fusarium Lini*, was more serious and caused severe damage to the crop.

Curves showing the number of surviving plants counted at weekly intervals are shown in Text-fig. 1. There was very heavy mortality at the pre-emergence and early seedling stage of development, germination being only about 30 % in the plots sown with treated seed and only 13 % with untreated seed as compared with a germination rate of 95 % in a neighbouring bed containing similar soil free from *F. Lini*.

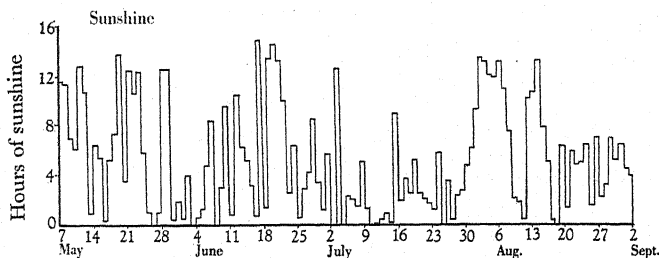
The difference between a germination rate of 95 % in one plot and 13 % in another plot of similar soil when both were sown with untreated seed may be ascribed primarily to the presence of *F. Lini* in the latter, but some allowance should be made for the fact that one bed was carrying a flax crop for the third successive year, whereas the other had not been sown with flax before.

The fungicides afforded a striking degree of protection during germination, and this lead over the untreated seed was maintained throughout

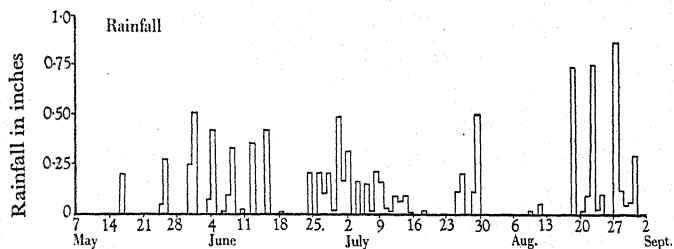
development. Analysis of the yields of the crops grown from treated and untreated seed showed that there was no significant difference in the number of flowering shoots on each plant, the number of capsules on each flowering shoot, or the number of seeds in the capsules, the higher yield from the treated seed being due entirely to the greater stand, (Pl. XIII. fig. 1).



(a)



(b)



(c)

Text-fig. 1. (a) Graph to show the relative stand of treated and untreated seed of the flax variety La Plata in wilt-infected soil in 1944. (b) Sunshine in hours during period of growth of 1944 crop. (c) Rainfall in inches during period of growth of 1944 crop.

Text-fig. 1 also shows the daily records for sunshine and rainfall while the crop was growing in 1944. A high mortality rate from wilt disease coincided with periods of dry, sunny weather. Greatest destruction occurred

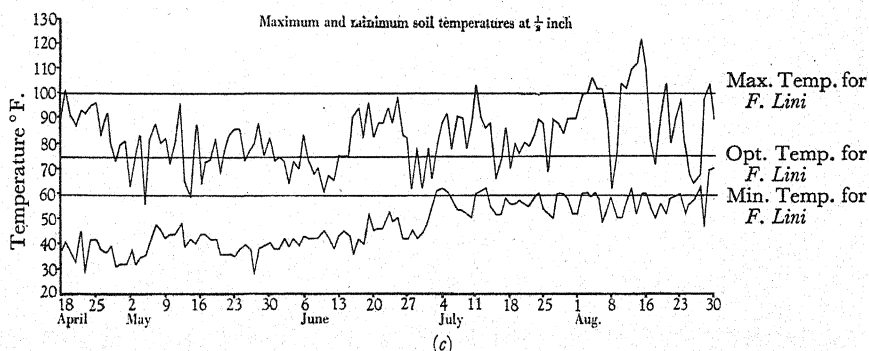
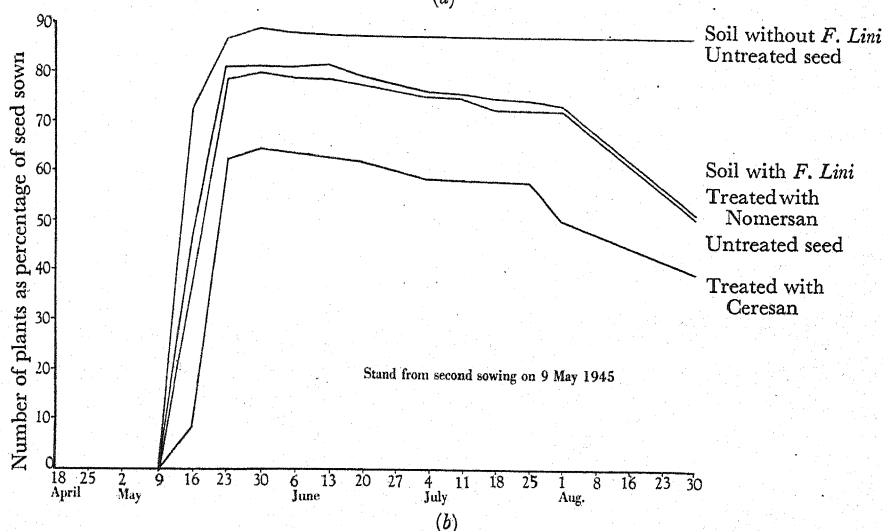
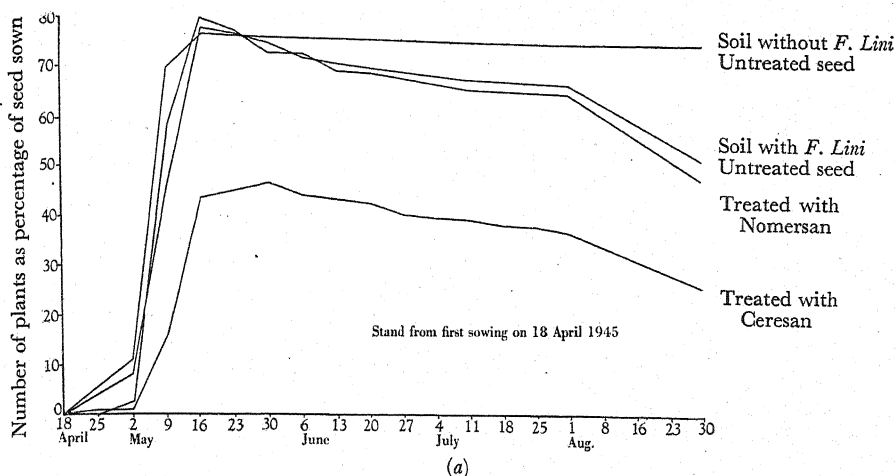
in the very early stages of germination, and there was another marked drop in the number of surviving plants during the 6th–7th week after sowing in the latter half of June, and again in the first part of August, but the older plants were not as rapidly killed as the younger ones and there was a short time lag. During periods of rather dry weather with bright sunshine, the soil temperatures are higher than in dull or wet weather. That a relatively high soil temperature is an important factor in favouring infection by *F. Lini* was shown by Jones and Tisdale (1922) and has been fully confirmed by many other investigators whose work has been summarized by Millikan (1945).

In 1945 an attempt was made to obtain more accurate information on the relation between soil temperature and infection by *F. Lini*, and for this purpose daily readings of the maximum and minimum soil temperatures were obtained at a depth of about half an inch, the level at which the infection of the plants generally occurs. At the same time, at the request of the Ministry of Supply, the fungicide Ceresan U 564 was tested, Nomersan also being used in other plots to give a standard of comparison.

The same infected soil, now in its fourth successive year under flax, was sown with La Plata seed which was free from all disease organisms except 0.5 % of *Polyspora Lini*. Plots were sown with untreated seed, seed dressed with 0.6 % Nomersan applied as a dry powder and seed treated with an 8 % solution of Ceresan U 564 by the short-wet method (Muskett & Colhoun, 1943). At the same time similar seed, not treated with any fungicides, was sown in another bed which had not previously grown flax and was therefore free from *Fusarium Lini*. Two sowings were made, the first on 18 April 1945 and another, three weeks later, on 9 May 1945. All the plots for each sowing were duplicated. The rate of sowing was approximately equivalent to fifty pounds per acre.

The weather was very cold and dry at the time of the first sowing, and during the first three weeks there were frequent ground frosts and occasional hail and sleet showers, but just before the second sowing the weather became warmer with showers and sunny periods, though frost occurred as late as 27 May 1945. As might have been expected, the seeds of the first sowing germinated much more slowly than those of the second sowing (Text-fig. 2), they showed more evidence of frost damage to the cotyledons, and the percentage of germination was lower. Nevertheless, the germination rate was much higher than it had been in the same bed the previous year, owing probably to the fact that the cooler weather was unfavourable to attack by wilt disease.

The curves (Text-fig. 2) representing the number of living plants in the two sowings, counted every week, show a striking parallelism as well as some significant differences. In both, there was no falling off in the stand of plants growing in the control plots free from *F. Lini*, except in the seedling stage and this was due entirely to *Polyspora Lini*. The number of living plants growing in soil infected with *Fusarium Lini*, on the other hand, showed a continuous decline which was particularly marked during the hot weather in August. The symptoms were similar to those for wilt disease, but, in addition, the incidence of disease due to *Polyspora Lini* was also



Text-fig. 2. (a) Graph to show relative stand of treated and untreated seed in wilt-infected soil and of untreated seed in healthy soil. First sowing, 18 April 1945. (b) As above. Second sowing, 9 May 1945. (c) Maximum and minimum soil temperatures at a depth of half an inch during period of growth of 1945 crop.

greater than in the control plots. Most of the older plants were infected with both fungi. Judged from the large number of isolations which were made, the *Polyspora* was at first confined to the leaves and to localized, superficial areas on the stem, while the *Fusarium* spread through the internal tissues of the stem and root. Since *Polyspora* infection alone did not prove fatal except for a few seedlings in the control plots, the main cause of the death of the plants in the other plots must be ascribed to *Fusarium Lini*. Some allowance must, however, be made for the decline in the fertility of the soil after four successive years under flax, and for the possible accumulation in the soil of other organisms inimical to flax.

The curves for the Ceresan treatment are very low in both sowings (Text-fig. 2). Colhoun (1945) has shown that treatment with Ceresan has the effect of lowering the percentage of viable seeds in subsequent storage, and that the higher the moisture content of the seed before treatment, the earlier this effect becomes apparent. These facts, no doubt, account for the results obtained in the present investigation where the moisture content of the seed before treatment was 8.1 % and the period in store was four to seven weeks. The test for Ceresan was therefore not a fair one. It has proved a valuable fungicide when the moisture content of the seed is low and the storage period is short (Muskett & Colhoun, 1943, 1944). That it provides particularly useful protection against stem break caused by *Polyspora Lini* was confirmed by a close examination of all the plants in all the plots five and eight weeks after sowing.

In both sowings, the results for the untreated seed and the seed dressed with Nomersan grown in wilt-infected soil are very close and follow the same general trend as the curve for Ceresan, but the falling off in the stand is a little steeper, probably on account of the greater incidence of infection with *Polyspora*, which in turn increases severity of attack by *Fusarium Lini*. There was a slight superiority in the germination of the seed treated with Nomersan in both sowings (Text-fig. 2), but this was not nearly so marked as in 1944. The weather in 1944 favoured attack by *F. Lini* in the early stages of germination, while in 1945 the weather was unusually cold in the early part of the summer, and the optimum conditions for infection did not occur until the plants were maturing, when seed dressings could not be expected to yield much protection.

Tisdale (1917) and Jones and Tisdale (1922) have shown that the critical soil temperature below which infection by *F. Lini* does not occur is about 57.2°F., that the optimum temperature for wilting lies between 75.2°F. and 82.4°F. and that infection does not occur above 96.4°F. These temperatures have been marked on the graph (Text-fig. 2) showing daily maximum and minimum soil temperatures at half an inch depth in the experimental plots, the lower value being taken for the optimum because work by Edwards (1945) suggests that this figure is the more accurate for the Aberystwyth isolates. Although the soil temperatures during 1945 were such that some infection would have been possible from the time of the first sowing, it is probable that the optimum temperature for infection was reached only for relatively short daily periods until at least the middle of June, and the soil temperatures remained low at night until the beginning of July. It was only during

the earlier part of August that the temperature requirements for infection by *F. Lini* fell squarely within the range of the maximum and minimum soil temperatures. The decline in the stand in the plots with soil containing *F. Lini* shows a distinct correlation with the soil temperatures, the higher temperatures leading to attack and destruction of the plants by this organism.

While wilt disease has developed each year from 1942 to 1945 in certain beds in the Botany Garden at Aberystwyth, the fungus has not spread in other beds with a different type of soil (Table 4) sown with infected seed.

Table 4. Comparison of two types of soil in Botany Garden (after successive cropping to flax without addition of fertilizers)

Soil		Loss on ignition	pH	Available phosphate	Available potash
Soil in which <i>F. Lini</i> spread:	A	14.5	4.21	0.0104	0.008
	B	17.5	4.25	0.0112	0.005
Soil in which <i>F. Lini</i> did not spread:	A	12.1	7.55	0.192	0.006
	B	12.4	7.96	0.192	0.008

The striking difference in the acidity of these soils was not the causal factor concerned in the spread of the fungus. Edwards (1945) has tested the Aberystwyth isolates on a pH range from 3.0 to 11.0 and has found that the growth is best on alkaline media with an optimum at pH 7.5. This is in part agreement with Anderson (1925), who found that the optimum was pH 5 in two cases and pH 7 in another, and with Bolley (1901*a*), who stated that the fungus makes most rapid growth upon a rich humus and seems to be most destructive on strongly alkaline land. The more important factor favouring growth of *F. Lini* through one of the soils was probably its higher organic content, which not only provided a rich nutrient substrate for the fungus, but in giving a much darker colour to the soil ensured a higher soil temperature, which on sunny days was up to 10° C. above that in the other type of soil.

Although the methods used in the present investigation are remote from standard agricultural practice, the main conclusion, that *F. Lini* may cause severe damage to the flax crop even under the conditions of the British climate, may be of interest.

SUMMARY

The occurrence and symptoms of wilt disease of flax caused by *Fusarium Lini* in experimental plots at Aberystwyth in 1942-5 are described. The source of infection was the Canadian seed of the variety Redwing.

Isolations of the fungus were made from seeds, stems and roots of diseased plants. Pathogenicity experiments using sterilized soil inoculated with these isolates showed that the fungus was a virulent parasite.

La Plata was very susceptible both in pathogenicity experiments and in the field. Redwing was fairly susceptible and Bison, although it suffered severely from the disease, was more resistant.

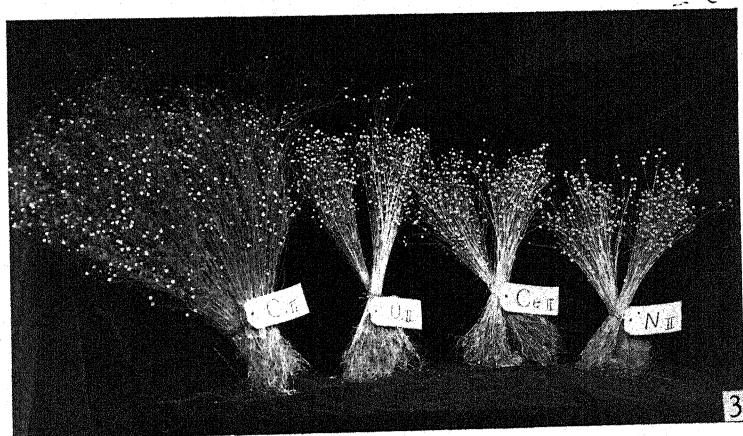
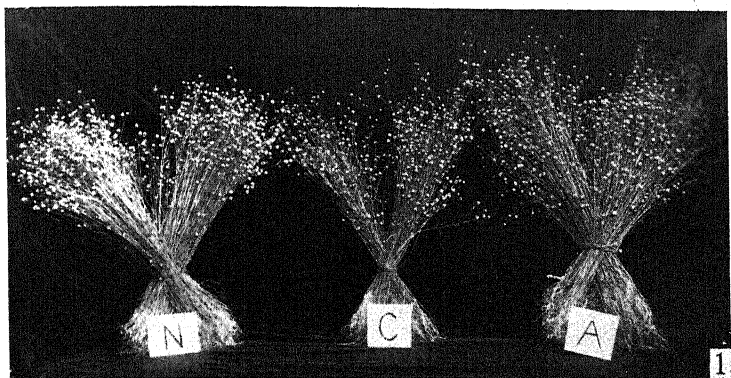
It was confirmed that flax plants can be attacked at any stage of development and that the incidence of wilt disease is closely correlated with suitable soil temperatures. When the plants were attacked in Aberystwyth, the disease was usually associated with infection by *Polyspora Lini*.

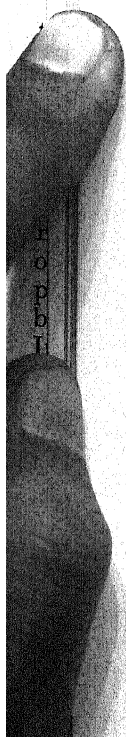
Seed dressings give considerable protection against infection during the early stages of germination, but are not so effective against attack on older plants. They may be a useful supplement to the more promising method of control, the breeding of resistant varieties of flax.

My thanks are due to Prof. G. E. Blackman for flax seed, to the Imperial Chemical Industries Ltd. for Nomersan and Agrosan G, and the Ministry of Supply for the fungicide Ceresan U 564 manufactured by Bayer Products Ltd., to Captain David Jones for meteorological data (except 27-30 May 1944), Mr R. O. Davies for the soil analysis and to Dr W. L. Gordon, Dominion Laboratory of Plant Pathology, Winnipeg, for the identification of the *Fusariums*.

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EXPLANATION OF PLATE XIII

- Fig. 1. Yields from plots of similar size in wilt-infected soil, 1944. *N*, seed treated with Nomersan; *C*, untreated seed; *A*, seed treated with Agrosan G.
- Fig. 2. Yields from two rows of first sowing, 1945. *C*, untreated seed in healthy soil; *U*, untreated seed in wilt-infected soil; *Ce*, seed treated with Ceresan U564 in wilt-infected soil; *N*, seed treated with Nomersan in wilt-infected soil.
- Fig. 3. Yields from two rows of second sowing, 1945. Lettering as for fig. 2.

(Accepted for publication 8 July 1946)

NOTE ON THE OCCURRENCE OF SPECIES OF *OIDIODENDRON* ROBAK IN BRITAIN

By G. SMITH, *London School of Hygiene and Tropical Medicine*

The genus *Oidiiodendron* was erected by H. Robak (1932) on three species which were all found growing on ground wood-pulp in Norwegian mills. The original three species are *O. nigrum*, *O. fuscum* and *O. rhodogenum*, the last being readily distinguished by its production of a blood-red diffusible pigment in wort agar cultures. Later, Robak (in Melin & Nannfeldt, 1934, p. 440) described a fourth species, *O. griseum*, and a further addition to the genus was made by von Szilvinyi (1941) with his *O. flavum*.

The genus is characterized by erect, arborescent conidiophores, with branching always monopodial, and by the differentiation of the terminal branches, from the tip inwards, into conidia. Superficially the fructifications resemble, except for their smaller size, those of *Cladosporium* (*Hormodendron*), but the method of spore production relates the genus to *Oidium* and *Torula* Pers.

So far as I can ascertain there have been, up to the present, no records of *Oidiiodendron* for Britain.*

Between 1923 and 1930 a considerable number of fungi, mostly Hyphomycetales, were collected for biochemical studies in the research laboratories of Nobel's Explosives Co. Ltd., at Ardeer, and in 1931 the collection of cultures was transferred to the London School of Hygiene and Tropical Medicine. Two of the cultures, both of which were catalogued as '*Hormodendron* or ?*Cladosporium*', obviously differed in cultural characteristics from typical *Cladosporium herbarum*, but no attempt was made to identify them until, recently, one of them was shown to have interesting biochemical properties.

Culture Ag 109. This was isolated by the late J. H. V. Charles from a lichen on a wooden post, in December 1926. It is a typical strain of *O. rhodogenum* Robak. Growth on all culture media is very slow, almost velvety or tufted floccose, pale grey, with overgrowth of dirty white sterile mycelium. On corn-meal agar or wort agar the whole mass of medium is gradually coloured blood-red. On other media pigment production is sparse and spasmodic.

Culture Ag 112. Isolated by the late J. H. V. Charles, April 1928, from gun cotton at Ardeer. Growth is very slow, grey to fuscous, powdery, with some dirty brown pigment in the medium. It agrees well with *O. fuscum* Robak.

Another strain of *O. fuscum* has recently been received from Dr A. Burges, Cambridge, amongst a batch of *Penicillium* cultures for identification. It was isolated in October 1945, from the surface humus in a pine plantation

* Since this Note was submitted for publication Dennis and Wakefield (*Trans. Brit. Mycol. Soc.* 1946, xxix, 141) have described a British strain of *O. fuscum* Robak.

at Wangford Warren, near Brandon. Dr Burges reports that he has, in addition, three other isolations from the same locality.

In view of the similarity of this genus, in gross appearance of the conidial fructifications, to *Cladosporium*, it seems probable that a search through the records of *Cladosporium* would bring to light other instances of the occurrence of species of *Oidiodendron* in Britain.

I am greatly indebted to Mr E. W. Mason, who identified the strain Ag 112 as *Oidiodendron*, and drew my attention to Robak's paper.

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(Accepted for publication 24 July 1946)

NOTES ON THE DIE-BACK OF OAK CAUSED BY *COLPOMA QUERCINUM* (FR.) WALLR.

By E. S. TWYMAN, *Department of Botany, University of Birmingham*

(With Plate XIV and 3 Text-figures)

1. INTRODUCTION

Colpoma quercinum (Fr.) Wallr. is a phacidiaaceous inoperculate, discomycete, causing a die-back of young oak trees, coppiced oak and the smaller terminal branches of older trees. Its taxonomy has been discussed by von Höhnelt (1917) and by Nannfeldt (1932). The brothers Tulasne (1853) and Brefeld (1891*a, b*) have described the associated conidia and Fries (1823), Rehm (1896), Neger and Dawson (1907) and Nannfeldt (1932) have described the ascocarp. The pathology of the fungus was dealt with by Frank (1880) and, in greater detail, by Neger and Dawson (1907).

2. THE ASCOCARP

Adequate photographs and drawings of apothecia, asci and ascospores do not exist beside descriptions of the species, and Pl. XIV, fig. 1, shows a few mature apothecia as they appear in May, June and July. They usually occur on dead twigs and branches, but occasionally they appear on the diseased side of a living branch. Nannfeldt (1932) describes the form of the apothecium, the hymenium of which consists of clavate asci ($130-150 \times 9-10 \mu$) with bluntly pointed apices and paraphyses with curled tips (Pl. XIV, fig. 2). The asci contain eight hyaline elongated spores ($15-115 \times 1.5 \mu$). The mode range of ascospore length is $40-65 \mu$. Rehm's (1896) measurements are $90 \times 1.5 \mu$.

The ascospores are violently discharged, a phenomenon previously observed in other discomycetes and pyrenomycetes by Jones (1925), Ingold (1928) and Buller (1909; 1934). The maximum height to which spores are discharged in still air is 14.5 mm., while most of them are ejected to heights up to 6.5 mm.

3. THE CONIDIA

(a) *Collected material*

The conidial apparatus has been described only in association with the ascocarps. It forms a melanconiaaceous acervulus, but it cannot be identified with any separately named member of the Melanconiaceae. Since observations of its structure do not precisely correspond with those of the brothers Tulasne (1853) and of Brefeld (1891*a, b*), a complete description is given.

While the acervuli appear on newly infected twigs before the apothecia, they are usually also closely associated with young apothecial rudiments. The shape of an acervulus is that of a truncated cone (Text-fig. 1 A) with a basal layer of pseudoparenchyma, 0.04-0.05 mm. in thickness. From

the centre of this basal layer the pseudoparenchymatous cone extends upwards towards the opening and sometimes protrudes through it (Text-fig. 1 A). This gives a bilocular appearance to a vertical section. Conidiophores (50μ long) arise from the basal parenchyma on each side of the core. Beneath the periderm, immediately above the conidiophores and conidia and extending from the opening to the edge of the basal parenchyma, is a thin web of loose hyphae (Text-fig. 1 A, C and D, *e*) which can be left in position by carefully removing the periderm. Under this thin layer of fungus tissue, the five to seven locules of the acervulus appear radially arranged round the dark brown central core of pseudoparenchyma (Text-fig. 1 B, C and D). The locules are delimited from the host tissue by a thin layer of black pseudoparenchyma. The conidia are extruded from the opening in the ruptured periderm as a cream or reddish globular mass.

(b) *Development in culture*

Oat sucrose, maize and oat agars are the best media for the formation of sporulating acervuli at room temperature. On these they appear after seven to eight weeks if the cultures are given an initial incubation at 25°C . for fourteen days.

The acervulus originates as a small hemispherical tuft of loosely interwoven hyphae ($1.0-2.0 \times 0.5-1.0\text{ mm.}$) the outer layers of which gradually become compacted to form a wall of pseudoparenchyma. This wall is differentiated into three regions: (a) a layer of loosely interwoven hyphae some of which are brown and $5-10\mu$ broad while others are finer and colourless (Text-fig. 1 F, *g*), (b) a layer of dense pseudoparenchyma formed of dark brown hyphae (Text-fig. 1 F, *h*), and (c) a layer of compact colourless hyphae that give rise to the conidiophores (Text-fig. 1 F, *k*). After seven or eight weeks the outer layers of the acervulus rupture (Text-fig. 1 G) and spores issue from the top as a cream, globular mass which turns red or pink.

(c) *The conidia*

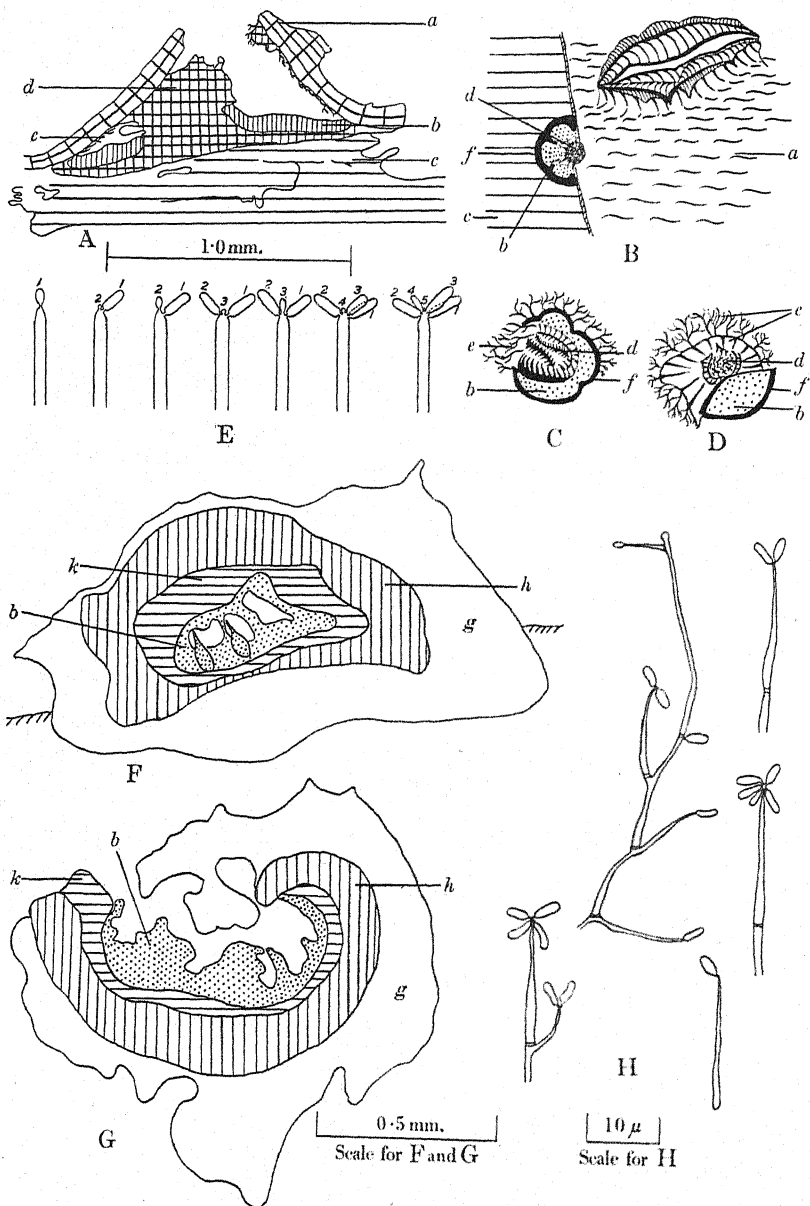
The conidia of *Colpoma quercinum* are unicellular, hyaline, cylindrical or slightly curved and rounded at each end. Measurements of the conidia given by three authorities are:

Brefeld	$7-8 \times 1.5\mu$
Saccardo	$8 \times 1.5\mu$
Tulasne	6.5μ

During this investigation 1600 conidia were measured. The lengths of spores produced on agar cultures had a mode range of $3.9-4.8\mu$ with a variation of $2.4-7.3\mu$. The mode range of the length of spores from naturally infected material was $4.4-5.3\mu$.

The disposition of the conidia on the conidiophores is described and figured by Brefeld (1891 *b*). My drawings are shown in Text-fig. 1 H. From observations, it is suggested that the conidia arise in basipetal succession, as shown in Text-fig. 1 E.

The conidiophores are very variable in length and may be branched or unbranched (Text-fig. 1 H). Sometimes numerous branched conidiophores



Text-fig. 1. A, diagrammatic vertical section through an acervulus of *Colpoma quercinum*: a, periderm; b, hymenium of conidiophores; c, infected cortex; d, pseudoparenchyma; e, thin web of mycelium. B, diagrammatic surface view of an acervulus and apothecium of *C. quercinum*; the periderm and underlying fungal tissue have been removed from half the acervulus: a, periderm; b, hymenium of conidiophores and conidia; c, infected cortex; f, black pseudoparenchyma limiting acervulus from host. C and D, diagrammatic surface views of two acervuli showing the thin covering of fungal tissue (e) over the hymenium; other letters as in A and B. E, diagram showing the origin of conidia at the tip of a conidiophore: 1-5, order in which conidia arise. F, diagrammatic section through an acervulus developed on agar: b, hymenium of conidiophores and conidia; g, loosely interwoven hyphae; h, dark compact pseudoparenchyma; k, compact hyaline pseudoparenchyma. G, diagrammatic vertical section through a ruptured acervulus developed in culture; letters as in F. H, group of conidiophores.

and sterile hyphae become grouped together, forming an elongated conical structure which terminates in a few sterile hyphae (Pl. XIV, fig. 3). This is the coremium-like structure referred to by Brefeld (1891 b).

Despite repeated attempts, the conidia were not germinated on any of the following media: distilled water, turnip extract, maize extract, oatmeal extract, plain agar, maize extract agar, and clear malt extract agar. This agrees with the findings of Brefeld (1891 a). Attempts to determine if these spores functioned as spermatia were unsuccessful.

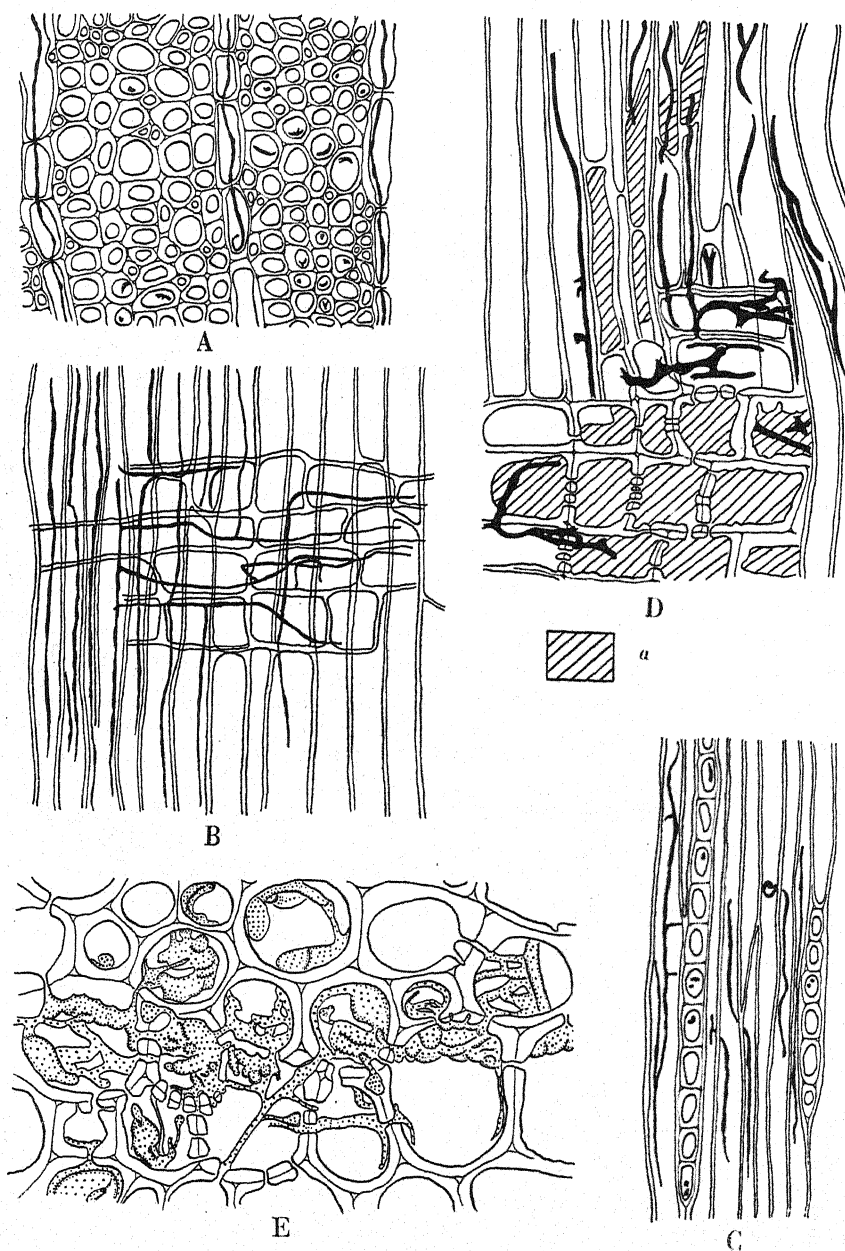
4. THE MYCELIUM OF *COLPOMA QUERCINUM* IN COLLECTED DISEASED WOOD

The distribution of mycelium in diseased wood was investigated by Cartwright's method (1929). Diagrams in Text-fig. 2 A, B and C show the appearance of typical mycelium in infected wood. The mycelium travels in a longitudinal direction in the vessels and tracheids and in a radial direction in the cells of the medullary rays. The direction in which the hyphae travel therefore seems to be determined by the shape of the cells in which they are growing. Black lines, formed by aggregated brown-walled, inflated hyphae filling the lumina of cells of the xylem and pith, are observed in the diseased wood (Text-fig. 2 E, and Pl. XIV, fig. 4). Similar lines produced by other fungi are described by Hiley (1919), Campbell (1934) and Wilkins (1936). Campbell considers the black line of *Armillaria mellea* to be the limiting edge of a sclerotium-like body immersed in the tissues of the host, but Wilkins (1936), working with *Ustulina*, found it associated both with the growing edge of the mycelium in the wood and irregularly distributed in the wood with no connexion with the growing edge. In *Colpoma quercinum* the black line was not associated with the growing edge of the mycelium, but was always found in diseased twigs bearing apothecia. If the periderm were removed, the line surrounded one or more apothecia (Pl. XIV, fig. 4), beneath which a pocket of diseased wood was found completely delimited by the black line. In longitudinal section the line descended radially into the wood and joined up with similar lines travelling in a longitudinal direction (Pl. XIV, fig. 4). These lines were not found within infected oak twigs (previously sterilized) bearing the conidial pustules. The line might be regarded therefore as the edge of a stroma bearing apothecia.

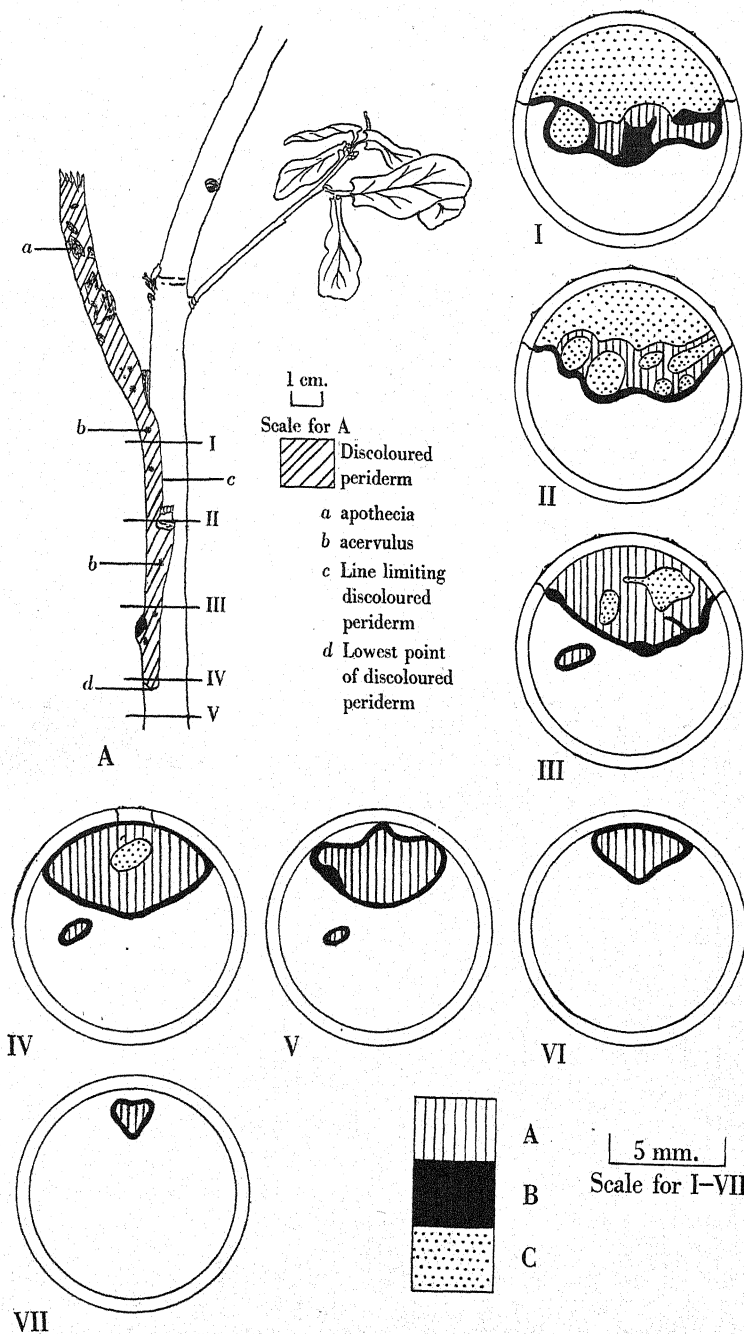
The mycelium of this fungus destroys the phloem and cortex, while the periderm remains free from attack. The relation between the mycelium in the xylem and in the phloem and cortex has yet to be determined.

5. THE PROGRESSION OF THE MYCELIUM FROM INFECTED TO HEALTHY BRANCHES

The actual mode of entry of *Colpoma quercinum* into the host is still a matter of conjecture, but there is evidence that infection takes place through wounds caused mechanically or by the depredations of insect larvae such as those of the moth *Tortrix viridana* (Neger & Dawson, 1907). The original diseased areas of this die-back usually occur on small twigs and the infection travels basally, entering the healthy parent branch and spreading towards



Text-fig. 2. A, transverse section of infected wood. B, radial section of infected wood. C, tangential longitudinal section of infected wood. D, radial section taken from light red brown region (see text): *a*, ligno-tannin complex. E, transverse section in the black line region of infected wood, showing the brown-walled, inflated hyphae. Hyphae black or stippled.



Text-fig. 3. A, diagram of infected lateral entering healthy main branch; I-V, positions where sections I-V were cut. I-VII, diagrams of transverse sections cut across twig A at points, 2.0, 4.3, 6.8, 9.0, 10.0 and 14.3 cm. respectively, below the junction of lateral with main branch; A, light red-brown region; B, dark red-brown region; C, pale highly infected region; sound wood is unshaded. (For further explanation see text.)

its apex and base. The latter progression of the disease in the host branch is most frequently encountered (Text-fig. 3 A). Several examples of this spread of infection have been examined and all conform to the same plan, so that a detailed account of only one of these will be given.

Text-fig. 3 A shows the external appearance of the specimen. The infected lateral branch bears apothecia, acervuli and discoloured periderm, while the diseased part of the main branch shows only acervuli and discoloured periderm.

As a result of an examination of seven transverse sections cut at intervals across the twig, four main regions were recognized: (1) sound wood (Text-fig. 3, I-VII, unshaded), (2) a narrow dark reddish brown region (Text-fig. 3, I-VII, B), (3) a wider lighter brown area (Text-fig. 3, I-VII, A), and (4) a pale region of highly infected wood (Text-fig. 3, I-IV, C). The consistency of the first three regions was hard, while that of the fourth was soft. The brown regions (Text-fig. 3 A and B), termed the gum barrier, cut off the highly infected area (Text-fig. 3 C) from the sound tissues. Microchemical tests on sections from the gum barrier showed the presence of lignin and tannin in the cells (Crocker, 1921; Haas & Hill, 1920). The brown substance was therefore termed a ligno-tannin complex. The difference in colour between the two brown regions was due to the relative proportion of cells filled with the ligno-tannin complex. Since no gum barrier was seen in uninfected twigs and as the greatest deposition of the complex is in the narrow region next to the sound wood, it may be assumed that the ligno-tannin complex is produced by the living tissues as a reaction to the advancing mycelium. The pale region (Text-fig. 3 C) contrasted with the gum barrier region in the softness and obvious decay of the wood. The difference between these two zones was attributed to the delignification of the cell walls and the digestion of the ligno-tannin inclusions in the cells of the infected tissues, since the microchemical tests for lignin and tannin gave negative results. Since hyphae were observed in some cells of the xylem containing the ligno-tannin complex, it is suggested that these products may be digested by the fungus (Text-fig. 2 D, a). Birkinshaw and Findlay (1940) give some indication of the method by which phenolic substances are digested.

6. SUMMARY

1. *Colpoma quercinum* (Fr.) Wallr. is an inoperculate, phacidiaecous discomycete, causing a die-back of oak trees. The conidia of this fungus develop very easily in culture and occur on an acervulus not previously described in the literature of the Melanconiaceae. The conidia do not germinate and no sexual function can yet be assigned to them.

2. The distribution of the mycelium of *C. quercinum* is such that the hyphae travel in a longitudinal direction in the vessels and tracheids and in a radial direction in the medullary rays. Black lines are formed which are probably the limiting edges of stromata bearing apothecia.

3. The natural progression of the mycelium of the fungus from infected to healthy branches is usually basal. A gum barrier laid down between infected and healthy wood contains a ligno-tannin complex.

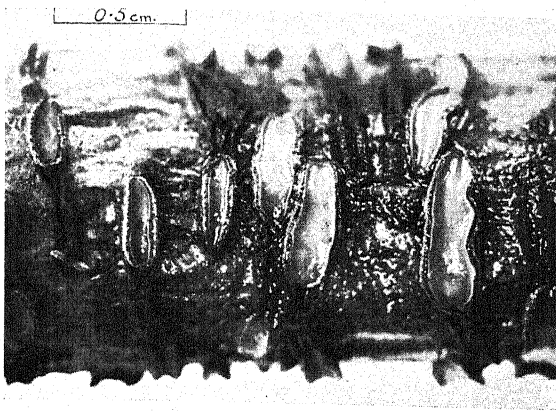


Fig. 1.

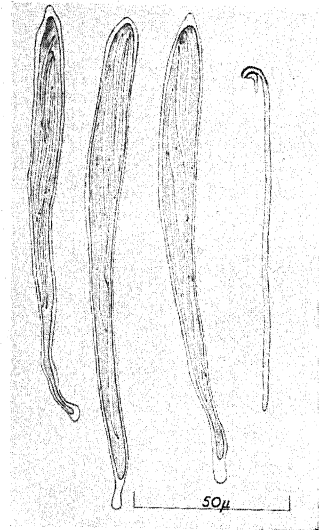


Fig. 2.



Fig. 3.

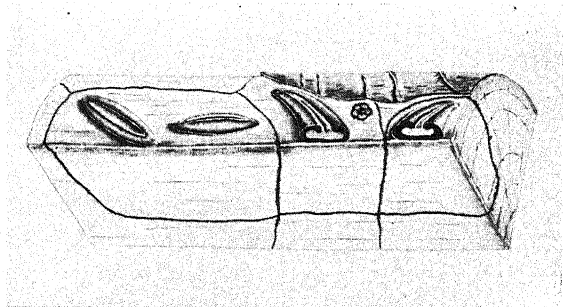


Fig. 4.

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EXPLANATION OF PLATE XIV

- Fig. 1. Mature apothecia of *Colpoma quercinum*.
- Fig. 2. Asci and a paraphysis of *C. quercinum*.
- Fig. 3. Coremial-like structure found in acervuli of *C. quercinum* developed in culture.
- Fig. 4. Part of a twig bearing apothecia, showing stromata limited by black lines.

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BIOMETRICAL RESEARCHES OF SECONDARY SPORES AND A STUDY OF THE MYCELIUM OF *PHOLIOTA* *ADIPOSA* FR., *P. HETEROCLITA* FR., *P. MUTABILIS* (SCHAEFF.) FR., *P. SPECTABILIS* FR. AND *P. SQUARROSA* (MULL.) FR.

By S. BATKO

(With 1 Text-figure)

I. INTRODUCTION

In the course of a study of secondary spores and the type of rot caused by species of *Pholiota* the results of some previous researches have proved very useful.

Cartwright (1929*b*) described the culture and secondary spores of *P. adiposa*. The decay and microscopical details of the mycelium of *P. squarrosa* on ash were recorded by Cartwright and Findlay (1942). Robak (1933) observed the fruit bodies of *P. mutabilis* in culture and described the type of rot produced by this fungus on wood pulp. Fruiting bodies of *P. heteroclita* on 2 % malt and of *P. squarrosa* were obtained on sterilized wheat grains by Cartwright and Findlay. Davidson, Campbell and Blaisdell (1938), confirming Bavendamms' hypothesis of the reaction of wood-decaying fungi grown on media containing small amounts of gallic or tannic acid, found that *P. adiposa* causes a white decay.

The writer's descriptions of the mycelia of species of *Pholiota* were based on those given by Snell (1922). The staining method for fungal mycelium described by Cartwright (1929*a*) proved very useful when hyphal measurements in wood had to be made.

II. ORIGINAL INVESTIGATIONS

For the studies described below, the following fungi were obtained from the Forest Products Research Laboratory, Princes Risborough, Bucks: *Pholiota adiposa*, 52 A; *P. heteroclita*, 159; *P. mutabilis*, 49; *P. spectabilis*, 126; and *P. squarrosa*, 90. The writer wished to carry out biometrical researches on secondary spores of these five fungi because, so far as he was aware, no previous work of this nature had been published. The following biometrical constants have been calculated for each: the mean, the standard deviation, the coefficient of variation and their standard errors.

In August 1945 the fungi were transplanted on 5 % Kepler's malt agar and 2 % agar in boiling tubes, and exposed to diffuse light in the laboratory. A few months later, both aerial and submerged mycelium were examined under the microscope and the following observations were noted. *Pholiota adiposa*, *P. mutabilis*, *P. squarrosa* formed abortive fruit bodies in culture. *P. spectabilis* formed typical fruit bodies (Fig. 1).

Pholiota adiposa

Aerial mycelium. Hyphae, secondary spores and oidia hyaline; clamp connexions present; secondary spores ellipsoidal, ovate, or sometimes almost orbicular; walls of spores thin; crystals present. The typical secondary spores and oidia were measured separately.

Submerged mycelium. Hyaline; secondary spores and oidia are found in the submerged mycelium, but not so profusely as in the aerial mycelium; clamp connexions rare; secondary spores; ellipsoidal, ovate, sometimes almost orbicular; very occasionally chlamydospores occur; crystals present.

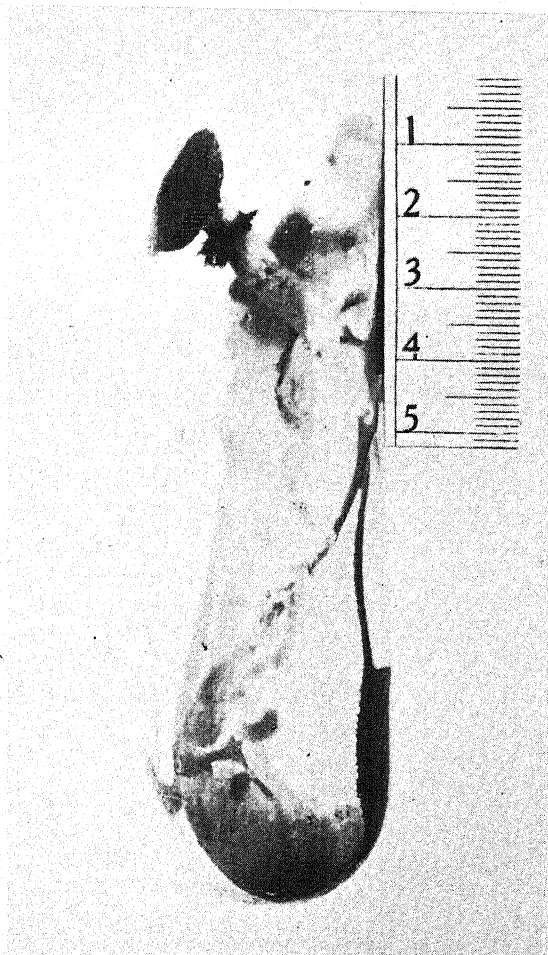


Fig. 1. *Pholiota spectabilis* fruiting on 5% malt, 2% agar after 4½ months.
The scale is given in cm.

Pholiota adiposa. Average size of secondary spores

Aerial mycelium				Submerged mycelium			
Length μ	No.	Breadth μ	No.	Length μ	No.	Breadth μ	No.
5	12	3	5	4	2	3	12
6	34	4	76	5	24	4	57
7	44	5	19	6	37	5	30
8	9	Total	100	7	24	6	1
9	1			8	11	Total	100
Total	100			9	1		
				10	1		
				Total	100		

Average size of oidia

Aerial mycelium				Submerged mycelium			
Length μ	No.	Breadth μ	No.	Length μ	No.	Breadth μ	No.
7	6	2	69	5	1	1.5	2
8	24	3	31	6	1	2	87
9	38	Total	100	7	11	2.5	5
10	26			8	20	3	6
11	6			9	28	Total	100
Total	100			10	33		
				11	5		
				12	1		
				Total	100		

Biometrical constants. Size of secondary spores

Length in μ			Breadth in μ		
Mean	Standard deviation	Coefficient of variation %	Mean	Standard deviation	Coefficient of variation %
A. Aerial mycelium					
6.5 ± 0.08	0.85 ± 0.06	13.1 ± 0.94	4.1 ± 0.05	0.47 ± 0.03	11.5 ± 0.82
B. Submerged mycelium					
6.2 ± 0.11	1.09 ± 0.08	17.6 ± 1.28	4.2 ± 0.06	0.64 ± 0.04	15.4 ± 1.14

Size of oidia

Length in μ			Breadth in μ		
Mean	Standard deviation	Coefficient of variation %	Mean	Standard deviation	Coefficient of variation %
A. Aerial mycelium					
9.0 ± 0.09	0.99 ± 0.07	11.0 ± 0.78	2.3 ± 0.05	0.46 ± 0.03	20.1 ± 1.47
B. Submerged mycelium					
9.0 ± 0.12	1.23 ± 0.09	13.7 ± 0.98	2.1 ± 0.03	0.27 ± 0.02	12.8 ± 0.92

Pholiota heteroclita

Aerial mycelium. Single hyphae hyaline, but yellowish in mass; clamp connexions frequent; no secondary spores formed.

Submerged mycelium. Hyaline; clamp connexions present, but not common; no secondary spores formed.

Pholiota mutabilis

Aerial mycelium. Single hyphae hyaline, but whitish in mass; secondary spores absent; crystals frequent and mainly within the hyphal cells.

Submerged mycelium. Hyphae hyaline; no secondary spores formed; crystals formed within and outside the mycelium; crystals less frequent than in the aerial mycelium.

Pholiota spectabilis

Aerial mycelium. Single hyphae hyaline, but whitish in mass; clamp connexions frequent; secondary spores numerous, almost orbicular in shape. About 50% of the spores have thick walls (approximately 1-2 μ in thickness); crystals formed both inside and outside the hyphae, but not frequent.

Submerged mycelium. Hyphae hyaline; secondary spores abundant, thin-walled; clamp connexions not abundant; crystals formed both inside and outside the hyphae.

Pholiota spectabilis. Average size of secondary spores

Aerial mycelium				Submerged mycelium			
Length		Breadth		Length		Breadth	
μ	No.	μ	No.	μ	No.	μ	No.
9	2	8	4	12	6	9	1
10	31	9	22	13	5	10	2
11	12	10	32	14	14	11	3
12	13	11	7	15	36	12	11
13	19	12	14	16	18	13	20
14	8	13	8	17	9	14	22
15	8	14	5	18	10	15	31
16	2	15	7	19	—	16	6
17	2	16	1	20	2	17	3
18	3	Total	100	Total	100	18	1
Total	100					Total	100

Biometrical constants. Size of secondary spores

Length in μ			Breadth in μ		
Mean	Standard deviation	Coefficient of variation %	Mean	Standard deviation	Coefficient of variation %
A. Aerial mycelium					
12.1 \pm 0.18	1.83 \pm 0.13	15.1 \pm 1.09	10.9 \pm 0.2	2.0 \pm 0.14	17.8 \pm 1.29
B. Submerged mycelium					
15.3 \pm 0.16	1.64 \pm 0.12	10.7 \pm 0.76	13.9 \pm 0.16	1.57 \pm 0.11	11.3 \pm 0.80

Pholiota squarrosa

Aerial mycelium. Single hyphae hyaline or yellowish; mycelium in the mass brown yellowish; secondary spores very rare; clamp connexions frequent.

Submerged mycelium. Hyaline; thin-walled secondary spores present; variable in form, usually pear-shaped, but others were ellipsoidal or orbicular, and some narrowed in the middle; clamp connexions present; crystals formed.

Pholiota squarrosa. Average size of secondary spores. Submerged mycelium

Length μ	No.	Breadth μ	No.
6	1	5	39
7	5	6	40
8	4	7	17
9	7	8	3
10	21	9	1
11	17	Total	100
12	16		
13	7		
14	5		
15	4		
16	2		
17	3		
18	3		
19	—		
20	2		
21	1		
22	1		
23	—		
24	1		
Total	100		

Biometrical constants. Size of secondary spores. Submerged mycelium

Length in μ			Breadth in μ		
Mean	Standard deviation	Coefficient of variation %	Mean	Standard deviation	Coefficient of variation %
11.9 \pm 0.33	3.31 \pm 0.23	27.9 \pm 2.12	5.9 \pm 0.09	0.86 \pm 0.06	14.6 \pm 1.05

THE MYCELIUM OF SPECIES OF *PHOLIOTA* IN BEECH WOOD

The beech blocks were put in the flasks containing the cultures of five *Pholiota* species and left exposed there to fungal attack for about four and a half months. Afterwards they were removed, sections stained with safranin and picroaniline blue and examined under the microscope. The following results were obtained:

(1) *Pholiota adiposa*. The blocks after removal from the flasks were found to be badly decayed. The rot was white, the timber being very soft, with mycelium distributed throughout. Seventeen sections were used for investigation, comprising radial, tangential and transverse sections. Twenty bore-holes dispersed in the various tissue were measured. The mean

dimensions are as follows: $6.7 \times 5.8 \mu$. Besides these, seven very large ones were observed. They measured: $90 \times 70 \mu$, $100 \times 75 \mu$, $70 \times 70 \mu$, $120 \times 80 \mu$, $110 \times 70 \mu$, $120 \times 110 \mu$, $110 \times 100 \mu$. Boring occurred both through the walls and pits. Clamp connexions occur. Diameter of hyphae $1-2 \mu$.

(2) *Pholiota heteroclita*. The cultures in flasks became contaminated and no records were made.

(3) *Pholiota mutabilis*. For microscopical researches thirty-five sections were used. The mean of eleven bore-holes is $4.1 \times 2.8 \mu$. There are, however, numerous very small bore-holes (about 1μ in diameter) and these have not been included in the general mean. The rot was white. Clamps were present. Hyphae from 1 to 3μ in diameter.

(4) *Pholiota spectabilis*. To describe mycelium twelve sections were used. The bore-holes were generally small (about 2μ in diameter), rarely larger bore-holes can be met with. The rot is white. Clamps present. Unlike the other four species of *Pholiota*, this fungus does not attack the pits to any appreciable extent. Hyphae $1-4 \mu$ in width. Secondary spores present. Some of them have thick walls.

Pholiota spectabilis. Average size of secondary spores. Mycelium in wood

Length μ	No.	Breadth μ	No.
7	1	7	1
8	—	8	1
9	6	9	16
10	17	10	40
11	20	11	22
12	18	12	15
13	24	13	3
14	9	14	1
15	4	15	1
16	1		
Total	100	Total	100

Biometrical constants. Size of secondary spores. Mycelium in wood

Length in μ			Breadth in μ		
Mean	Standard deviation	Coefficient of variation %	Mean	Standard deviation	Coefficient of variation %
11.8 ± 0.16	1.65 ± 0.12	14.1 ± 1.01	10.5 ± 0.12	1.24 ± 0.09	11.9 ± 0.85

(5) *Pholiota squarrosa*. In order to obtain accurate figures, the holes were separately measured for each type of cell. Thirty-two sections were used:

- (a) *Vessels*. The mean of eighteen bore-holes was $8.0 \times 6.4 \mu$.
- (b) *Fibre tracheids*. The mean of twenty-two bore-holes was $6.7 \times 5.4 \mu$.
- (c) *Fibres*. The mean of twenty-two bore-holes was $3.9 \times 2.5 \mu$.
- (d) *Medullary rays and parenchyma*. The mean of ten bore-holes was $6.8 \times 5.8 \mu$.

The general mean (for all types of cell) $6.3 \times 5.0 \mu$. The numerous small bore-holes ($1-2 \mu$ in diameter), especially in fibres and parenchyma, have

not been included in the general mean. Clamp connexions occur, occasionally proliferating. Hyphal diameter $1-3\mu$. Boring through walls and pits. Mycelium uniformly distributed throughout. The colour of the rot is white.

METHOD OF DETERMINING SIGNIFICANCE OF RESULTS

(1) *Significance of differences between means.* The significance of the differences has been checked by the following formula:

$$\frac{m_1 - m_2}{\sqrt{(\sigma_1^2 + \sigma_2^2)}} (A).$$

m_1 and m_2 are the mean values to be compared. σ_1 and σ_2 are standard errors of two means. $\sigma_1^2 = s_1^2/n_1$, $\sigma_2^2 = s_2^2/n_2$, s_1 , s_2 are standard deviations of the groups from which the means are derived. n_1 , n_2 are number of results in two groups, here $n_1 = n_2 = 100$. If the ratio (A) exceeds 2, it may be considered that the difference is significant; if the ratio is below 2, then the difference is not significant, but is such as might reasonably have been obtained from repeated sampling in a single population only.

CONCLUSIONS

Fungus	Mean size of secondary spores					
	On aerial hyphae		On submerged hyphae		On hyphae in wood	
	Length μ	Breadth μ	Length μ	Breadth μ	Length μ	Breadth μ
<i>P. adiposa</i>						
Secondary spores	6.5	4.1	6.2	4.2	—	—
Oidia	9.0	2.3	9.0	2.1	—	—
<i>P. spectabilis</i>	12.1	10.9	15.3	13.9	11.8	10.5
<i>P. squarrosa</i>	—	—	11.9	5.9	—	—

Pholiota adiposa. The difference between the size of secondary spores of the aerial and submerged mycelia is not significant.

The difference between the size of oidia of the aerial and submerged mycelia is not significant.

Pholiota spectabilis. The difference between the size of secondary spores of the aerial and submerged mycelium is significant.

The difference between the size of secondary spores of the aerial mycelium and the mycelium in wood is significant.

The difference between the size of secondary spores of the submerged mycelium and the mycelium in wood is significant.

Pholiota adiposa and *P. spectabilis.* The difference between the size of secondary spores of the aerial mycelium of *P. adiposa* and *P. spectabilis* is significant.

The difference between the size of secondary spores of the submerged mycelium of *P. adiposa* and *P. spectabilis* is significant.

Pholiota adiposa and *P. squarrosa.* The difference between the size of secondary spores of the submerged mycelium of *P. adiposa* and *P. squarrosa* is significant.

The difference between the size of secondary spores of the aerial mycelium of *P. adiposa* and of the submerged mycelium of *P. squarrosa* is significant.

Pholiota spectabilis and *P. squarrosa*. The difference between the mean length of secondary spores of the aerial mycelium of *P. spectabilis* and of the submerged mycelium of *P. squarrosa* is not significant.

The difference between the mean breadth of secondary spores of the aerial mycelium of *P. spectabilis* and of the submerged mycelium of *P. squarrosa* is significant.

The difference between the size of secondary spores of the submerged mycelium of *P. spectabilis* and *P. squarrosa* is significant.

The difference between the mean length of secondary spores of the submerged mycelium of *P. squarrosa* and of the mycelium in wood of *P. spectabilis* is not significant.

The difference between the mean breadth of secondary spores of the submerged mycelium of *P. squarrosa* and of the mycelium in wood of *P. spectabilis* is significant.

(2) Secondary spores of *P. squarrosa* are very variable in shape and in length. They have the largest coefficient of variation of the length of all species investigated.

(3) The following is the arrangement of the fungi according to size of bore-holes (beginning with the largest): (1) *P. adiposa*, (2) *P. squarrosa*, (3) *P. mutabilis* and (4) *P. spectabilis*.

SUMMARY

The secondary spores of five *Pholiota* spp. are described and the statistical significance of their differences in size is discussed.

I am much obliged to Dr F. Y. Henderson, Director of Forest Products Research, for kind permission to carry on research in his Laboratory, to Miss Pettifor of the Timber Mechanics Section and to Messrs K. St G. Cartwright and W. P. K. Findlay for valuable suggestions, and to Mr E. C. Badcock for kind help and assistance during my work in the Mycological Section.

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NEW AND INTERESTING PLANT DISEASES

By W. C. MOORE, *Plant Pathology Laboratory, Harpenden*

(With Plates XV-XVII)

25. MILDEW (*Oidium Valerianellae* Fuckel) ON CORN SALAD

In November 1945 and again in the spring of 1946 Dr Katharine H. Johnstone kindly sent me specimens of corn salad (*Valerianella olitoria* Poll.) growing in her garden at Helland Bridge, near Bodmin. The plants had been raised from seed of an unnamed variety, probably of North American origin. Some of the lower stem leaves were brown and withered, and bore pycnidia of a species of *Phyllosticta*, while on the younger leaves there were patches of mildew which, to the naked eye, strongly suggested a downy mildew, though on closer examination it proved to be a species of *Oidium*. The mildew was very sparse on the specimens sent on 9 November and 26 March but, on those received 10 April 1946, when the plants had 'bolted' and begun to flower, it was present as conspicuous white, fluffy flecks on the upper surfaces of the leaves. It was also fruiting, though less obviously, on the undersides of the foliage leaves, on the stems, and on the small bracts surrounding the flowers. The conidiophores were simple, often grouped in small tufts, and bore chains of up to at least six long-elliptic, oblong, or barrel-shaped conidia, ranging in size from $27-39 \times 13-21 \mu$ (average of thirty conidia $32.8 \times 17.8 \mu$).

No powdery mildew has been recorded in Britain on *Valerianella*, but in August 1943 Dr P. H. Gregory gave me some specimens of a mildew that he had found on most of the plants in an acre of *Valeriana officinalis* grown in Hertfordshire. Numerous cleistocarps of *Erysiphe Cichoracearum* DC. were present on the affected leaves. There was no sign of a perfect stage on the corn salad, and the mildew can conveniently be identified with *Oidium Valerianellae* Fuckel (*Symb. mycol.* 1869, p. 358), which has been recorded on *Valerianella carinata* in Germany and Italy and on *V. Morisonii* in Germany. Lindau, in *Rab. Krypt. Fl.* 1, viii (1904), p. 87, suggested that this species may be merely a form of *Oidium erysiphoidea* Fr., which is listed in Oudemans' *Enum. Syst. Fung.* iv, 853, on several species of *Valerianella*, including *V. olitoria*. Blumer (1933) has also given a number of European localities for the *Oidium* stage of what he believed to be *Erysiphe communis* (Wallr.) Fr. on *Valerianella olitoria* Poll., *V. dentata* Poll. and *V. rimosa* Bast., including two records listed by Salmon (1900) under *Erysiphe Polygoni* DC. but not verified by him.

26. MILDEW (*Oidium* sp.) ON *SCHIZANTHUS*

There are only two or three records of a powdery mildew on *Schizanthus* and apparently none from outside Britain. In December 1934 a species of *Oidium* was observed on the leaves of one or two plants in a small greenhouse at the Plant Pathology Laboratory, Harpenden (Moore, 1943). The

mildew was very slight, difficult to detect, and was causing no damage. It was not seen in England again until April 1945, and I am indebted to Mr N. C. Preston for specimens of an *Oidium* which he then found on *Schizanthus* in flower in a private greenhouse at Sutton Coldfield, Warwickshire. The attack was a severe one and the lower leaves had been killed. The *Oidium* was present in quantity, but there was no sign of cleistocarps: the barrel-shaped conidia measured $19-34 \times 11-16 \mu$ (average of twenty conidia $25.8 \times 12.8 \mu$) in the dry state. The mildew reappeared in the same greenhouse in the spring of 1946.

Erysiphe Cichoracearum DC. has been listed on this host from the Forth area of Scotland (Dennis & Foister, 1942), but Dr Foister has informed me in a letter that in the absence of confirmatory evidence he would prefer this record to stand as *Oidium* sp.

27. LEAF SPOT (*CERCOSPORA DEPAZEOIDES* (DESM.) SACC.) OF
ELDER (*SAMBUCUS NIGRA* L.)

In September 1941 Dr Alex Smith and I found a leaf spot causing partial or complete defoliation of many rather small shrubs of elder (*Sambucus nigra* L.) in a comparatively open oak-beech wood at Symonshyde, near Harpenden. The spots were numerous, visible on both sides of the leaves, and mostly 2-5 mm. across. They were rounded, or bounded by the secondary veins and then irregular, uniformly dull grey when small, later becoming bleached dirty white or pale brown, usually with a dull grey border. On many leaves the spots had coalesced, but the darker margins of the original spots remained as grey lines running in wavy patterns over the affected areas. Conidia of *Cercospora depazeoides* (Desm.) Sacc. (*Nuovo G. bot. ital.* viii (1876), 187, syn. *Exosporium depazeoides* Desm. in *Ann. Sci. nat.* (3), xi (1849), 364) were present on both sides of the spots, though more freely produced on the lower surfaces. They measured $50-147 \times 4-6 \mu$ (average of twenty-five spores $89 \times 4.9 \mu$) and showed 1-6 transverse septa.

The following month the disease, which had apparently not been recorded previously in Britain, was observed a few miles away at Wheatthampstead (Herts), and Dr H. B. S. Montgomery sent me specimens from woods near East Malling (Kent), where the disease was causing complete defoliation of some of the trees. It was also found during a foray at Cooper's Hill (Surrey) in September 1942 and is perhaps not uncommon in England.

28. BLACK ROT OF ANEMONE (*SCLEROTINIA TUBEROSA* (FR.) FUECKEL)

This disease was carefully studied by Wakker (1889) in Holland, and fifty or sixty years ago it was a subject of frequent correspondence in the English gardening press (*Gdnrs' Chron.* 1886-1903), but very little has been heard of it during the past thirty years. Black rot shows certain unusual and interesting features. It attacks the native *Anemone nemorosa*, but has been found in the past mainly in gardens and rockeries on cultivated varieties of *A. nemorosa*, as well as on *A. coronaria*, *A. apennina*, *A. blanda* and *A. ranunculoides*. It may be destructive to pot plants (Burchard, 1929), but is

seldom seen in commercial plantations. It has not yet been recognized, for instance, in field crops in Cornwall where over 700 acres were devoted to Du Caen and St Brigid anemones in 1938. This is perhaps the more surprising because the large sclerotia of *Sclerotinia tuberosa* so closely resemble the corms of *Anemone coronaria* that it is very difficult to separate them. Although very destructive, the disease has attracted most attention because of the extraordinary abundance of the coffee-coloured apothecia produced from the sclerotia in April and May. As many as forty apothecia may arise from a single sclerotium (Thomas, 1902), and W. G. Smith (1887) wrote: 'when this fungus grows in *Anemone* beds in gardens it does not grow in single examples; it grows in hundreds, or, in large flower-beds, it sometimes appears in thousands of examples.' In the only recorded outbreak of black rot in this country in recent years (May 1933) very large numbers of apothecia appeared at Harborne, near Birmingham, in a rock garden where few of the anemones escaped attack.

A fresh phase of the disease came to light in October 1941 when I received from a nursery at Taplow (Bucks.) some rhizomes of *A. nemorosa* var. *Robinsoniana* which had been completely destroyed by *Sclerotinia tuberosa* during storage. They had been lifted the previous August and stored in bulb trays kept in racks in such a way as to permit good ventilation. As usual, the rhizomes had been buried in peat in the trays to prevent them from shrivelling. When examined on 10 October the long, thin, light brown, stick-like rhizomes were still fresh and healthy in several of the trays, but in two trays there remained nothing but a congealed mass (Pl. XV, fig. 1) consisting of discoloured brittle fragments of rhizomes and a number of the large black sclerotia characteristic of *S. tuberosa*, the whole being matted together and enveloped in a loose web of white mycelium. In a third tray the rot was just beginning. It seems very probable that a few of the rhizomes were slightly affected with black rot before they were put into the trays. The sclerotia were black, irregular, rounded, hard bodies up to $1\frac{1}{4}$ in. across and nearly $\frac{1}{2}$ in. thick (Pl. XV, fig. 2), white inside, and consisted of a mass of interlacing hyphae $4-6\mu$ wide, surrounded by a narrow black rind $15-40\mu$ thick. A few of them were placed in a pot of soil and left outside during the winter but, as is apparently not unusual, no apothecia appeared the following spring.

S. tuberosa is closely allied to *S. bulborum* (Wakk.) Rehm, the cause of black slime of hyacinth and *Scilla*, but its sclerotia are much larger, the apothecia are produced much more freely and have larger, deeper brown discs, and there are other slight morphological differences. Moreover, the fungus on hyacinth will not attack anemone and that on anemone apparently will not infect hyacinth.

29. A VIRUS DISEASE OF COS LETTUCE (DANDELION YELLOW MOSAIC VIRUS)

Kassanis (1944) proved that a virus which commonly produces chlorotic rings and spots on dandelion leaves is also the cause of a lettuce disease that had been observed in different parts of England during the previous three years. He briefly described the symptoms artificially induced in glasshouse

lettuce by this virus, for which he suggested the name dandelion yellow mosaic virus.

A disease, later shown by Dr Kassanis to be caused by this virus, appeared in mid-July 1942 on some Cos lettuce plants (variety Balloon) which had been raised under glass and pricked out for summer use in two adjacent gardens in Harpenden. It was seen first in one small clump in my own garden, at a time when the healthy plants were just beginning to heart, and by the end of the month it was present in several other clumps not many feet away, but developed in only one plant in the adjacent garden. The visible effect on the plant was not always the same, and appeared to vary with the time at which infection took place.

Several plants, evidently infected when quite young, were only 6 in. instead of a foot high (Pl. XVI, fig. 3). The outer four or five leaves were small but otherwise normal, with no obvious mottling or chlorosis. The next inner leaves were small and narrow, crimped or puckered, and showed a marked general necrosis which began as minute, rather angular, pale brown interveinal dots that later coalesced to give patterns of dark brown, grey-brown or almost black tissue immediately bordering the veins, even the finest ones (Pl. XVI, fig. 4). The necrosis was often particularly noticeable in the middle part of the leaves. The margins of some leaves were wilting or even withered, while in other leaves the tips and sometimes the margins for $\frac{1}{2}$ cm. inwards had a burnt appearance. The youngest leaves showed no visible necrosis.

Infected plants with outer leaves green, turgid, and a foot long, showed rather different symptoms, though it was always the next inner leaves which were mainly affected. Sometimes these leaves were pale and partly withered from the tips or margins inwards. The tissues were then brown, dry and papery, or the interveinal areas had fallen out, leaving holes. The veins usually showed a marked brown or black streaking. In other plants there were a few scattered inconspicuous pale brown areas on the outer mature leaves, while the next inner ones showed only a local or diffuse and rather ill-defined necrosis, the most prominent symptom being a ladder-like brown fretting of the outer, and occasionally the inner, surface of the main and lateral veins. The youngest leaves rarely showed more than a faint necrosis.

It is highly probable that this lettuce disease has been present in England for some time. Salmon & Ware (1934) referred to the occurrence of an unidentified disease in Balloon lettuce at Lancing (Sussex) and in the Cos variety, Mammoth White, at Scaynes Hill (Sussex) in July 1933, while specimens of a similar attack in black-seeded Balloon lettuce were received from King's Lynn in September 1938. Photographs and information about these cases, filed at the Plant Pathology Laboratory, strongly suggest that this virus disease was involved. Since 1942 it has been observed by Mr L. Ogilvie at Long Ashton, Somerset (July 1945) and by Mr H. H. Stirrup in a number of outdoor crops in the Kesteven area of Lincolnshire (June 1946). Dandelion yellow mosaic virus has been transmitted artificially by *Myzus ornatus* Laing, *M. pseudosolani* Theob. (Kassanis, 1944), and *M. ascalonicus* (Doncaster & Kassanis, 1946), but not by *M. persicae* Sulz.

30. LETTUCE RUST (*PUCCINIA OPIZII* BUBÁK)

Towards the end of May 1930, during a special examination in the London markets of lettuces imported from Holland, rust was found in very small quantity on the outer leaves of several plants. Only about a dozen rust spots were seen altogether. Each was about $\frac{1}{2}$ in. across and consisted of clusters of aecidia and spermogones. There was no hypertrophy of the tissues. The same rust, which was identified at the time as *Puccinia Opizii* Bubák (Pethybridge, Moore & Smith, 1934), has since been observed on two other occasions in lettuce imported into this country from Holland. A small quantity was seen on the London market in June 1932, and in May 1946 a few aecidia were found by Mr R. N. Swanton on the outer leaves of lettuces in four out of ten crates examined on the quayside at Newcastle-on-Tyne. It has not yet been observed occurring naturally on either wild or cultivated lettuce in Great Britain.

There are two aecidial forms on wild species of *Lactuca* in Europe. One is a stage of the autoecious rust, *Puccinia Chondrillae* Corda (also called *P. Prenanthis* (Pers.) Lindr. and *P. Lactucarum* Syd.), which is found, probably in specialized forms, on *Lactuca muralis* and *L. perennis*. This aecidium has a poorly developed peridium and causes slight hypertrophy of the leaf veins. The other is a stage of the heteroecious rust, *Puccinia Opizii* Bubák, with its aecidia on various species of *Lactuca*, *Crepis*, *Sonchus*, and on *Lapsana communis*, and its uredospores and teleutospores on *Carex muricata*. The aecidia have a complete and well-developed peridium and form larger spots, with no hypertrophy of the leaf tissues.

Bubák (1902) erected the name *Puccinia Opizii* when he showed experimentally that *Aecidium lactucinum* Lagerh. & Lindr. on *Lactuca muralis* and *L. scariola* belonged to the life cycle of a *Puccinia* on *Carex muricata*. Later, Tranzschel (1909, 1914) and Mayor (1920) proved that the rust on *C. muricata* can develop its aecidia on other species of *Lactuca*, including *L. sativa*, as well as on *Lapsana communis* and several species of *Crepis* and *Sonchus*.

Puccinia Opizii is still comparatively rare on cultivated lettuce in Europe and appears to be more or less innocuous. In Germany it was recorded in 1892 near Berlin, under the name *Aecidium Lactucae-sativae* Syd. (Sydow, 1924), and has also been observed in Brandenburg (Pape, 1926) and the Rhineland (Poeverlein, 1940). Poeteren (1933) has referred to its occasional occurrence on lettuce in Holland and there are records of it from Poland (Siemaszko, 1929) and Rumania (Săvulescu, Sandu-Ville, Rayss & Alexandri, 1934).

The aecidium occasionally found on lettuce in North America appears to be a different one. Arthur (1912) produced aecidia on *Lactuca sativa* and *L. canadensis* with a *Puccinia* from *Carex siccata* Dewey which he called *Puccinia Opizii* Bubák, but Kern (1917) concluded that all the American forms of rust with aecidia on the closely related genera *Adopogon*, *Agoseris*, *Crepis*, *Lactuca*, *Hieracium* and *Prenanthes*, and with uredospores and teleutospores on various species of *Carex*, including *C. siccata*, belonged to the same species, and one which was not identical with the European species. He

accepted for it the name *Puccinia patruelis* Arthur (1909), syn. *P. Opizii* Arth. non Bubák and *P. extensicola* Plowr. var. *hieraciata* (Schw.) Arth. This rust has been recorded on cultivated lettuce from seven Canadian provinces (Crowell & Lavalee, 1942) and in small amount from North Dakota (Sprague, 1945). A rust has also been listed on lettuce in New South Wales (Noble, Hynes, McCleery & Birmingham, 1934) under the name *P. prenanthis* (Pers.) Lindr.

31. RADISH CANKER (*CORTICIUM SOLANI* (PRILL. & DELACR.) BOURD. & GALZ.)

Corticium Solani, in its mycelial stage *Rhizoctonia Solani* Kühn, is a common cause of damping off, wire stem, and other disease symptoms in many different kinds of plants, including crucifers. It sometimes attacks radishes, and on this host most frequently produces blackish, canker-like areas on the maturing 'root', which have been illustrated abroad by Gloyer & Glasgow (1924), Chupp (1925), and Weber (1932).

Radish canker has been observed only a few times in Britain. It was first recognized on specimens received at Harpenden in June 1928 from a farm at Taplow, Bucks. *Corticium Solani* was isolated in pure culture and the blackish rot was reproduced by inoculation into the few rather old and stringy radishes that were then available. The same month a disease that was very similar in appearance proved rather serious and destructive in a market garden crop of long red radishes near Preston, Lancs., but no mycelium could be found in the rotted tissues and attempts to isolate *C. Solani* were unsuccessful. In June 1935 the disease was reported to have developed in patches in two farm crops on sandy soil at Mortlake (Surrey) and on heavier soil at Bedfont (Middlesex). Specimens received from Mortlake showed dark, soft, canker-like lesions on the sides or towards the top of the swollen edible portion of the root and no difficulty was experienced in isolating *C. Solani* from the advancing margin of the rotted tissue, which contained an abundance of mycelium. When healthy radishes were inoculated with the fungus from pure culture and were placed under moist conditions, rotting set in after a day or two and progressed so rapidly that within a fortnight only the discoloured skin and part of the vascular network remained more or less intact. Naturally infected radishes ultimately rotted in the same manner. Mr W. Buddin, who visited the farm at Mortlake, estimated that no more than one plant per thousand was affected, but this was sufficient to spoil the appearance of the bunches, and it was not possible to discard the affected radishes, partly because the lesions often escaped detection until the roots were washed. Mr Buddin also visited the farm at Taplow where canker had been seen seven years before and he had no difficulty in finding the disease; it was said to occur there every year but to cause no serious trouble. Too frequent cropping with radishes was thought to be a predisposing factor.

Preliminary tests indicated that strains of *C. Solani* isolated from potato would not infect radish. The radish strain failed to infect carrots, parsnips, mangolds and potatoes inoculated late in July and kept under moist con-

ditions, but white turnips and radishes inoculated the same day were completely rotted by mid-August. These results were later confirmed and extended by Storey (1941) who tested thirteen strains of *C. Solani* from various hosts and found that those from cruciferous plants were restricted in their attack to cruciferous hosts. His radish strain, derived from Harpenden, did not attack lettuce, potato or tomato, but fairly readily infected seakale, swede and seedlings of stock (*Matthiola*).

Gloyer & Glasgow (1924) have reported effective control of radish canker by several applications of acidulated mercuric chloride.

32. SEEDLING DISEASE OF FLAX (*ALTERNARIA LINICOLA* GROVES & SKOLKO)

On 9 June 1942 Mr W. Buddin sent me some diseased flax seedlings about 6 in. high, which had been taken from a field of linseed at Binsted, Hampshire. The same trouble also occurred in a field on a second farm sown from the same bulk of untreated seed.

At first sight the seedlings appeared to be healthy except for the cotyledons, which were brown and withered and fell off at a touch (Pl. XVII, fig. 5). Most of the affected cotyledons were partially or wholly blackened owing to the presence on them of numerous tufts of conidiophores and conidia of a species of *Alternaria* with long-beaked spores. Closer examination revealed that on some seedlings there were minute dark spots on the first pair of foliage leaves above the cotyledons. The spots spread irregularly to give necrotic areas 1–2 mm. across, and on a few seedlings this pair of leaves was completely discoloured and shrivelled. There was also an occasional pale brown streak on the stem just above the cotyledons and more frequently a scarcely perceptible shallow, canker-like area on the hypocotyl or at the base of the main root. The rim of the canker was reddish brown, and reddish brown streaks extended from it upwards into the hypocotyledonary region. The *Alternaria* was successfully isolated in pure culture from the cotyledons and from some of the minute spots on the foliage leaves, but not from the roots. It is undoubtedly the same species as the recently described, seed-borne fungus *A. linicola* Groves & Skolko (1944), syn. *A. linicola* Neergaard (1945). In the thirty spores measured the spore body was 30–100 μ long and 11–27 μ wide (average $65 \times 16 \mu$), with 4–11 transverse septa. The beak was 15–135 μ long (average 60 μ) and the total length of the spores 60–235 μ (average 125 μ). Dr Mary Noble has informed me that in 1945 she found *A. linicola* Groves & Skolko on flax seed at Edinburgh, and later obtained it from seedlings in two fields in Perthshire sown with the infected seed.

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EXPLANATION OF PLATES XV-XVII

- Fig. 1. Stored rhizomes of *Anemone nemorosa* var. *Robinsoniana* destroyed and matted together with mycelium and sclerotia of *Sclerotinia tuberosa*.
Fig. 2. On the left a rhizome of *Anemone nemorosa* var. *Robinsoniana* killed by *Sclerotinia tuberosa*: on the right a single sclerotium of the fungus.
Fig. 3. Dwarfed lettuce plant infected with dandelion yellow mosaic virus.
Fig. 4. A single infected lettuce leaf showing interveinal necrosis.
Fig. 5. Flax seedlings showing cotyledons and first foliage leaves attacked by *Alternaria linicola*.

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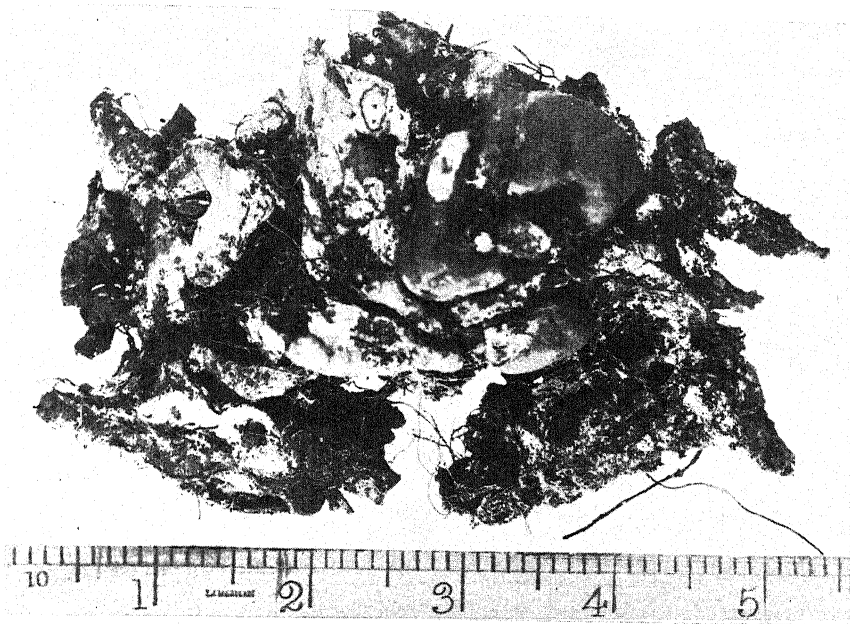


Fig. 1.

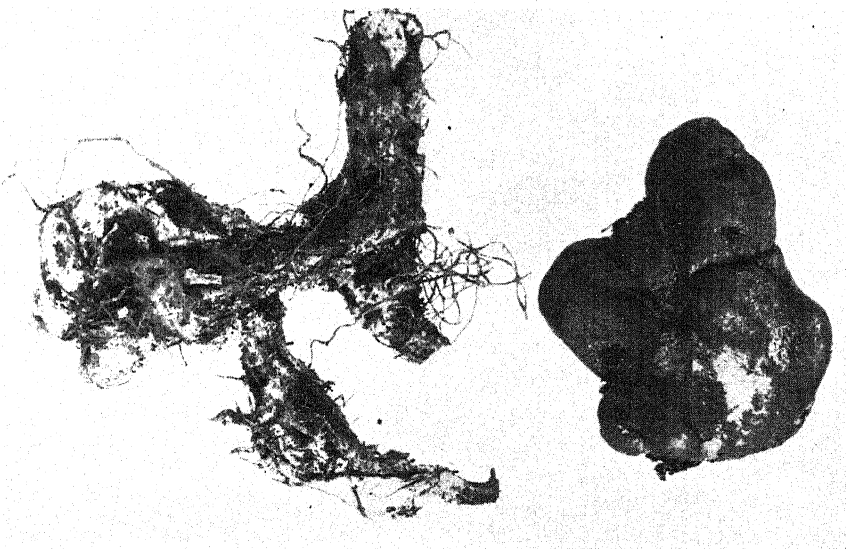


Fig. 2.



Fig. 3

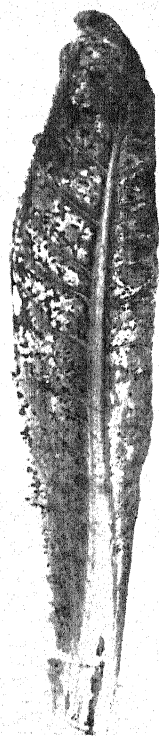


Fig. 4



Fig. 5

REVIEWS

The Mycetozoa of North America, based upon the specimens in the Herbarium of the New York Botanical Garden. By ROBERT HAGELSTEIN, Honorary curator of Myxomycetes. New York: Robert Hagelstein, 165 Cleveland Avenue, Mineola. 1944. Cloth, 306 pages, four colour plates and twelve in half-tone. Price \$6.00.

The Mycetozoa have attracted many students in North America, and no doubt its vast terrain, with large stretches of forest and swamps, and wide range of climatic conditions form admirable grounds and supply habitats for a large number of species of this interesting group.

The Mycetozoa of North America is a welcome addition to the literature of these organisms and is the result of a great amount of field work by the author and his associates over a period of twenty years. They have studied in the field and collected more than ten thousand developments. In addition, as curator of the Myxomycetes in the Herbarium of the New York Botanical Garden, Mr Hagelstein has had access to a large amount of material collected by earlier workers on the group in his own country and also that donated by workers in many parts of the world.

It is interesting that he has adopted the name Mycetozoa in preference to that of 'Slime Moulds' used by previous North American writers, and thus comes into line with the term used in this country and elsewhere.

The book describes 285 species and fifty-one varietal forms found in North America and thus deals with most of the Mycetozoa of the world. Only thirty-three species are quoted as not having been found in that country. It is well printed in good clear type and contains four coloured plates; the author refers the reader for all other illustrations to the British Museum *Monograph of the Mycetozoa*, Lister, 3rd ed., which cannot be surpassed. The plan of printing the names of species in heavier type than the synonyms is to be commended and facilitates reference. In addition, there are a number of plates in half-tone made from photographs by the author. It is not easy to produce good photographic records of Mycetozoa and in my opinion some of the sporangia could well have been shown larger and with more depth of focus.

The introduction is very good, and it is interesting to learn that fruiting usually takes place at night. The instructions to would-be collectors are very helpful, but I prefer to use somewhat deep tin boxes with a sheet of cork fastened to the bottom, especially when a gathering is made of a species whose development is not completed. The substratum on which the species is forming is usually moist and there is less risk of quick drying in a tin than in a cigar box lined with corrugated paper.

Mr Hagelstein does well to emphasize the fact that the group provides great opportunity for research in morphology, physiology and taxonomy; there are several problems yet unsolved. In the glossary a plasmodium is defined as 'a mass of naked protoplasm formed by the union of many swarm cells which latter emerge from the spores on germination'. This definition requires considerable qualification, the plasmodium being actually formed from amoebulae which unite in pairs as gametes to form zygotes. It is these zygotes which, after feeding, develop into plasmodia. However, in the introduction, this phase of life history is more clearly explained.

With regard to taxonomy, the author's descriptions are extremely well done and his judgements are very sound. One would have liked to have known more of the seasonal distribution of many species.

Some changes have been made from the previously accepted nomenclature in Lister, 3rd ed. Mr Hagelstein, strictly following the International Rules of Nomenclature, according to which a synonym must not be used again on any fresh association, substitutes *Physarum Listeri* Macbr. for *P. luteo-album* Macbr., as *P. luteo-album* Schumacher is now considered to refer to *Perichaena corticalis* (Batsch) Rost. For a similar reason *Physarum Bilgramii* replaces *P. lilacinum* Sturgis & Bilgram, as *P. lilacinum* Fries (1829) is now *Badhamia lilacina* Rost. *P. Wingatense* replaces *P. columbinum* Sturgis, and similarly

Didymium anomalum appears as *D. Sturgisii*. Since Miss Lister agrees that the genus *Trichamphora* is somewhat artificially separated from the unwieldy genus *Physarum*, the change of *Trichamphora pezizoidea* Jungh. to *Physarum pezizoideum* appears to be justified.

It is of interest that whereas *Physarum carneum* G. Lister & Sturgis occurs on dead wood and twigs in North America, I found it for four years always under dense bramble thickets upon bramble stems and dead bracken.

Physarum notabile Macbr. (syn. *P. connatum* List.) is a fine example of Mr Hagelstein's critical work in the determination of a species, and the same remark applies to his analysis of other groups of allied species, e.g. *Lepidoderma Carestianum*, its var. *granuliferum*, and *L. Chaillatii*.

In giving some varietal forms specific rank, as in the separation of *Stemonitis trechispora* Torrend (Macbr.) from *S. fusca*, because it has an extremely wide range of capillitium and spores, and a different habitat, is it necessary to separate it from *S. fusca*? Is it not possible, as the author stresses in his conclusion, that the varietal forms here and elsewhere may be due to a change of habitat, just as differences of temperature appear to cause a species to diverge from its typical form? The same remark appears to apply to the separation of the two varietal forms of *Didymium nigripes* Fries, giving them specific rank as *D. eximium* and *D. xanthopus*. Miss Lister states that these have been distinguished by specific names, but says that the variation in colour of capillitium, stalk, and columella occurs in the same group of sporangia and that they seem to blend into one another so completely as to represent one species. Both the type-form and var. *xanthopus* have been frequently met with by me in Norfolk and elsewhere and there does not seem sufficient reason for their separation. Mr Hagelstein himself states that somewhat reluctantly he has treated the three as distinct species and admits that they seem to occur when wet and moist conditions of habitat are reversed.

Stemonitis ferruginea Ehrenb. has been discarded in favour of *S. axifera* (Bull.) Macbr. in spite of the reasoned argument of Miss G. Lister (in Lister, 3rd ed.), in favour of its retention. Mr Hagelstein admits that *Comatricha aequalis* Peck, to which he gives specific rank, is practically the same as *C. nigra* Schroet., with similar capillitium and spores, differing only in the larger sporangia. He further states that many students have considered it as a varietal form of *C. nigra* and with some reason, as it is not common and occurs only occasionally where that species is abundant. It therefore seems difficult to understand why he separates it from *C. nigra* var. *aequalis* Sturgis as in (Lister, 3rd ed.)

Enerthenema papillatum Rost. has been split up into three species, the type form *E. papillatum* Rost., *E. melanospermum* Macbr. & Martin, and *E. Berkeleyanum* (Rost.) var. *syncarpon* (Sturg.) G. Lister. The author doubtless had access to several gatherings and he states that the three species are sharply defined.

In dealing with *Lamproderma atrosporum* Meylan, the typical form of which has not been recorded from Britain, the varietal forms var. *anglicum* G. Lister & Howard and var. *debile* G. Lister & Howard are omitted, not having been found in U.S.A., but the author suggests that they are intermediate forms connecting the species with *L. cribrarioides* and *L. Gulielmae*. I would like to point out that both forms were gathered in 1918, 1920, 1922, and 1923 on dead beech and box leaves under clumps of *Buxus sempervirens* in precisely the same spot by me, and Mr E. Brazier also found them on beech leaves in Worcestershire. *Lamproderma cribrarioides* has been recorded only from Aberdeenshire and typical *L. Gulielmae* has only once been recorded from Norfolk by me. The fact that the two varietal forms were met with over a space of five years seems to negative any idea that they must have been due to climatic conditions during development.

Macbrideola scintillans Gilb. n.gen. found on bark of living trees is an interesting addition to the list of arboreal species so well studied by the late Rev. Cran in Scotland. *Elaeomyxa myzakiensis* (Emoto) Hagelstein, found in Japan, adds a new genus to the Mycetozoa.

In dealing with *Cribraria aurantiaca* Schrad., the author quotes the plasmodium as green or slate-grey (Lister). In 3rd ed. Miss Lister gives the colour of the plasmodium of *C. vulgaris* Schrad. (1797) as usually slate-grey, whereas in var. *aurantiaca* Pers. (1801) it is green. Both forms have been seen in all stages by me in abundance on coniferous wood, and by the rules of priority *C. vulgaris* should be the name for the species and *C. aurantiaca* (as stated in Lister, 3rd ed.) with longer stalks, golden-yellow spores when fresh, and maturing from bright green plasmodium should be given varietal rank.

Cribraria microcarpa (Schrad.) Pers. as found in Europe represents a form different from that found in N. America; might not this be true of other species whose specific rank has been challenged? In Lister, 3rd ed., *Orcadella parasitica* (Zukal) Hagelstein (1942) appears as *Hymenobolina parasitica* (Zukal). The similarity of habitat on dead bark, mosses, lichens, and its general character seem to justify its inclusion in the genus *Orcadella*. The same justify the transfer of *Kleistobolus pusillus* Lipp. to the genus *Orcadella*, especially in view of the large amount of material which was available for examination. In his description of *Arcyria cinerea* (Bull.) Pers. var. *digitata* (Schw.) G. Lister, Hagelstein includes the varietal form but states that it is hardly worth recognition as a variety, much less as a species. I have made many gatherings of this common species in the type form, but as the variety appears to be confined to the tropics, where it is abundant, I think Mr Hagelstein does well to retain it as a definite variety.

Arcyria magna Rex. has been given specific rank. In Lister, 3rd ed., it is regarded as a form of *Arcyria Oerstedtii* Rost.; possibly in view of the number of gatherings both of *A. Oerstedtii* and *A. magna* in North America, the separation may be justifiable in spite of the fact that *A. magna* does not occur in any particular area.

The book concludes with a very comprehensive bibliography, and the author is to be congratulated on having produced a work which should attract many more students to the study of a fascinating and interesting group and which can be regarded as the standard work upon the Mycetozoa of North America.

It is with deep regret that shortly after this review was written the news came that Mr Hagelstein died on 20 October 1945. His passing represents a great loss to the somewhat small band of workers throughout the world on the group to which he devoted so much time and painstaking work.

H. J. HOWARD

The Aquatic Oomycetes of Wisconsin. Part I. By FREDERICK TAYLOR WOLF, Vanderbilt University. (Madison: The University of Wisconsin Press, 1944.) 64 pp., 6 plates. Price \$1.50.

Dr Wolf writes in the opening words of his introduction: 'Of all the aquatic fungi, perhaps none are more common or widely distributed than those of the family Saprolegniaceae.' It is because of the wide distribution of these and other 'Water Moulds' that this book, intended primarily for Wisconsin students, is of more than local interest, and workers in this field of study are indebted to Dr Wolf for the list of aquatic oomycetes given with useful keys and short, clear descriptions of each order, genus, and species, for which descriptions he acknowledges the value to him of Coker's monograph on the Saprolegniaceae.

The book opens with an interesting historical survey beginning even before the researches of de Bary. Then follows the list of species grouped in the Lagenidiales, Blastocladales, Monoblepharidales, Saprolegniales, Leptomitales, and Pythiales.

Where one would like to enter into discussion with Dr Wolf about his book is on questions of taxonomy. Why, for instance, does he, himself a student of the Blastocladales, include this order among 'Oomycetes', an order in which the oospore is conspicuous only by its absence and where the characteristic resting sporangium is the dominating feature? It is true he states that his key to the orders is 'after Fitzpatrick, 1930', but Prof. Fitzpatrick would be the first to disclaim it now, on the basis of work published since 1930. Again, why are the Lagenidiales separated from the Saprolegniales by the Monoblepharidales? Since 1930, Dr Sparrow has published an acceptable re-grouping of these orders on the character of the flagellum (which Dr Wolf still calls the cilium). Again, the only reference to *Phytophthora* in the book is on p. 44 in brackets: '(*Phytophthora*, a non-aquatic genus, has conidia [*sic*] which may either release zoospores or germinate by germ tube).' The explanation of this omission is to be found on p. 49 under *Pythiomorpha*: 'Buisman (1927) proposed to unite this genus with *Phytophthora*, and in this he [*sic*] was followed by Fitzpatrick (1930). We prefer, however, to follow Kanouse and retain *Pythiomorpha* as a valid genus of the Pythiaceae.' The insecurity of this argument is evident in the description of *Pythiomorpha Fischeriana* which runs: 'Sexual organs were not observed, but the asexual structures easily [*sic*] served to

identify the fungus. The cymose mycelium and conspicuous apically-papillate sporangia are quite distinctive.' Evidently all the *Phytophthora*-like aquatic fungi found in Wisconsin will be named as species of *Pythiomorpha*.

The illustrations have the value of diagrammatic drawings in their clear outline and emphasis upon essentials, but some of their disadvantages too. For instance, in figures of the species of *Pythiomorpha*, those supposed diagnostic characters of papilla and sporangio-phore are omitted. By the way, the shape of this sporangium is ob-pyriform not pyriform.

But, in spite of these criticisms of taxonomy, terminology, and illustration, the value of the straightforward account of species found and identified cannot be questioned. A later edition will give opportunity for correction of a few minor errors in the text, and, it is hoped, may include additional ecological data.

This slim volume is attractively produced by the University of Wisconsin Press. The neat shape and size and the good print on good paper make the book very pleasant to hold and read. And the lettering on the spine is the right way up when the book lies on its back on the table.

E. M. BLACKWELL

THE TWENTIETH ANNUAL PLANT PATHOLOGY FIELD DAY

By invitation of Dr J. Grainger the Twentieth Plant Pathology Field Day was held in the Department of Plant Pathology, West of Scotland Agricultural College, Auchincruive by Ayr, on Saturday, 29 June 1946. About forty members were present in the Plant Pathology Laboratory at 10.30 a.m. when Dr Grainger welcomed the Society and gave an interesting talk, illustrated by exhibits, on the work that his Department was doing on the relation between climatic conditions and plant diseases. After an animated discussion on this subject Mr H. F. Dovaston gave an account of his investigation of *Fusarium* Foot Rot of Cereals, with particular reference to the influence of external conditions on the pathogenicity of the species of *Fusarium* he was studying. Members then paid a brief visit to some of the experimental plots near the laboratory and spent further time in examining the exhibits. Dr Grainger commented on demonstrations which illustrated problems that had arisen from advisory work, and Mr Booth described the work he had begun on frost damage in that area.

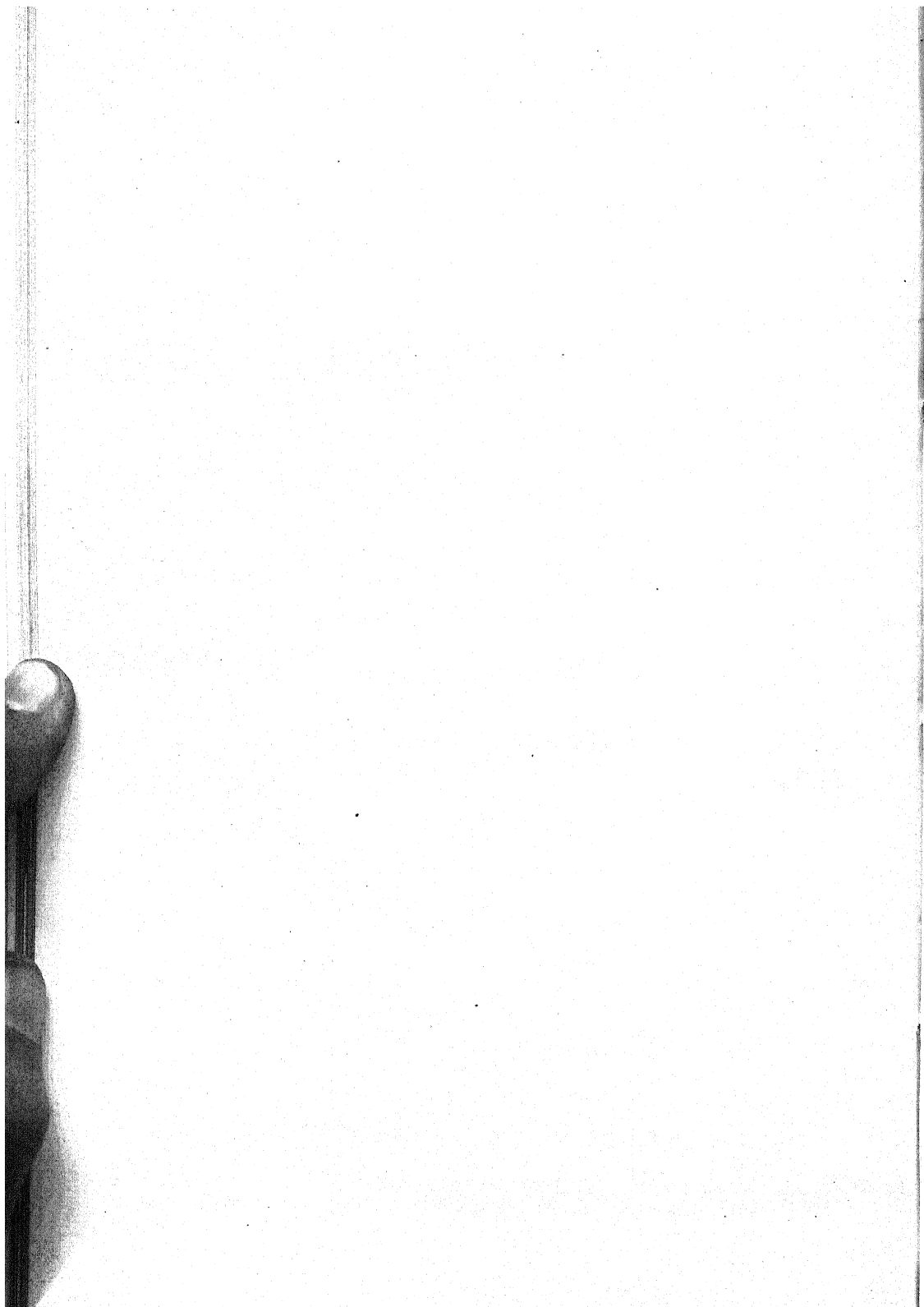
The members were the guests of the Governors of the West of Scotland Agricultural College at lunch. After lunch the Chairman of the Plant Pathology Committee, Mr W. C. Moore, thanked the Governors and Prof. Walton for their kind hospitality and expressed the appreciation of the Society to Dr Grainger and his colleagues for arranging such an interesting programme. He also specially welcomed to the meeting the Rev. Robert Barr, whose association with the Society went back to its earliest years.

Members then moved to the Horticultural Department where Mr R. D. Reid described his work on the breeding of strawberry varieties resistant to Red Core (*Phytophthora Fragariae* Hickman). This was followed by a lively discussion after which, in spite of heavy rain, some members visited Mr Reid's field plots while the remainder examined the strawberry plots near the Department. After tea and more informal discussion at the Hostel the members dispersed at about 4.30 p.m.

H. E. Croxall, *Secretary*
Plant Pathology Committee

NOTICE

Dr R. K. S. Wood, of the Imperial College of Science and Technology, South Kensington, London, S.W. 7, would be grateful to receive samples of mouldy paint from any source, together, where possible, with details of the kind of paint, location of painted surface, and approximate atmospheric conditions (greenhouse, bakery, brewery, etc.), and the address of the sender.



INDEX

New names are indicated by an asterisk

- Abies* (fir), 195
Achlya Nees, 135
 americana Humphrey, 103
 bisexualis Coker, 106
 colorata Pringsh., 106
 de Baryana Humphrey, 106
 flagellata Coker, 106
 polyandra Hildeb., 106
Adopogon, 254
Aecidium Lactucae-sativae Syd., 254
 lactucinum Lagerh. & Lindr., 254
Aegopodium Podagraria, 153
Agaricus campestris L., var. *pratensis*, vapor-
 arius Vitt., 202
 lanuginosus Bull., 199
Agoseris, 254
Agropyron repens, 159
Agrostis, 161, 162, 165
Ainsworth, G. C., 'Notes on the taxonomy and
 nomenclature of British smuts,' 189
Aira, 60
 flexuosa, 59
Allium, 90, 91
 ascalonicum (shallot), 90
 Cepa (onion), 90, 163
 lanceolatum, 90
 oleraceum, 92
 Porrum (leek), 90
 sativum (garlic), 90
 schoenoprasum (chives), 90
 vineale, 90, 92
Alnus (alder), 97
Alternaria Nees ex Wallr., 78-84, 185, 256
 Anagallidis Raabe, 162
 linicola Groves & Skolko, 256
 linicola Neergaard, 256
 Porri Neergaard, 163
 solani (Ell. & Mart.) Jones & Grout, 163
 tenuis Nees ex Wallr., 78, 81
Amanita abietinum Gilbert, 192
 **pantherina* DC., f. *robusta* A. A. Pearson,
 n.f., 191
 phalloides (Vail.) Fr., 108
 rubescens (Pers.) Fr., 55, 58, 59
Amanitopsis fulva Sacc., 55
 vaginata Fr., 60
Anagallis arvensis (scarlet pimpernel), 162
 var. *caerulea*, 163
Anellaria separata (Linn.) Karst., 55, 56
Anemone, 251
 apennina, 251
 blanda, 251
 coronaria, 251, 252
 nemorosa, 251
 var. *Robinsoniana*, 252
 ranunculoides, 251
Angelica sylvestris, 34
 Annual General Meeting, 1945, 95
Anthostoma turgidum (Pers.) Nits., 97
Anthoxanthum odoratum, 160
Anthriscus sylvestris, 13, 31, 32
Anthurus Kalchbr., 141
 Archeri (Berk.) Fischer, 141
Antirrhinum, 163
Apium graveolens, 153
Aposphaeria Berk., 12
Arcyria cinerea (Bull.) Pers., var. *digitata*
 (Schw.) G. Lister, 261
 magna Rex, 261
 Oerstedtii Rost., 261
Armillaria mellea (Vahl) Fr., 60, 237
Ascochyta Lib., 13, 21, 26, 39, 40
 linicola Naumoff & Vassilievski, 13, 26
 lycopersici Brun., 27
 pinodella L. R. Jones, 39
 pirina (Fr.) Pegl., 40
Ascochyta Grossulariae Died., 38
Ascotremella Seaver, 152
 faginea (Peck) Seaver, 152
Aspergillus Mich. ex Fr., 5
 amstelodami (Mangin) Thom & Church, 5
 fumigatus, Fres., 211, 214
 mut. *helvola* Yuill., 211
 niger van Tiegh., 5, 214
Aspidium, 158
 Filix-femina, 144
Avena byzantium, 165
 sativa (oats), 83, 164, 165
Bacterium Ehrenb. em. Dowson, 185
Badhamia lilacina Rost., 259
 Barnes, B. Review: 'Die Hefesammlung des
 "Centraalbureau voor Schimmelcultures". Bei-
 träge zu einer Monographie der Hefearten.
 II. Teil. Die anaskosporogenen Hefen.
 Zweite Hälfte', by H. A. Diddens &
 J. Lodder, 100
 Batko, S. 'Biometrical researches of secondary
 spores and a study of the mycelium of
 Pholiota adiposa Fr., *P. heteroclita* Fr., *P.*
 mutabilis (Schaeff.) Fr., *P. spectabilis* Fr.,
 and *P. squarrosa* (Müll.) Fr.', 242
 Beta (beet, mangold, sugar beet), 115, 255
 Betula (birch), 60, 97, 191, 199, 206, 208
 Bhargava, K. S. 'Oogenesis and fertilization
 in *Isoachlya anisospora* var. *indica*', 101
 Biometrical researches of secondary spores and
 a study of the mycelium of *Pholiota*
 adiposa Fr., *P. heteroclita* Fr., *P. mutabilis*
 (Schaeff.) Fr., *P. spectabilis* Fr., and *P.*
 squarrosa (Müll.) Fr., by S. Batko, 242
 Blackwell, E. M. Review: 'The Aquatic
 Oomycetes of Wisconsin, Part I', by F. T.
 Wolf, 261
Boletus auriporus Peck, 206
 badius Fr., 59
 Carpini (R. Schulz) A. A. Pearson, 207, 208
 chrysenteron (Bull.) Fr., 55, 60
 cramesinus Secr., 206
 duriusculus Schulz, 208

- Boletus (*cont.*)
 edulis (Bull.) Fr., 55, 59
 erythropus (Pers.) Quél., 59
 leucophaeus Pers., 207
 pseudo-scaber Kallenb., 207
 sanguineus (With.) Fr., 206
 var. *gentilis* Quél., 206
 scaber Auctt., 206, 207, 208
 scaber (Bull.) Fr., var. *Carpini* R. Schulz,
 207
 var. *coloripes* Singer, 208
 subtomentosus (Linn.) Fr., 59
 tenuipes (Cooke) Massee, 206
 Bomarea, 92
 Bond, T. E. T., 'Natural occurrence of
 sprouting of the ascospores of *Sphaerulina*
 Mappiae (Petch) n.comb., in Ceylon', 86
 Botryosporium longibrachiatum (Oud.)
 Maire, 97
 pulchrum Corda, 97
 Botryotinia Whetzel, 150
 Botrytis (Mich.) Link, 150
 Allii Munn, 173, 175, 176, 182, 183, 185,
 186, 188, 213
 cinerea Fr., 150, 221
 convoluta Drayton, 150
 Bouteloua gracilis, 165
 Boyd, A. E. W., Foister, C. E. & Wilson, A. R.,
 'Infestation of soils by *Fusarium caeruleum*
 in relation to the incidence of dry rot in
 potatoes', 189
 Brassica (swede turnip, turnip), 15, 16, 256
 Sinapis, 23
 Brettanomyces Kuff. & v. Laer, 100
 Brian, P. W., 'Fungistatic substances produced
 by *Trichoderma viride*', 189
 'Production of gliotoxin by *Penicillium*
 Terlikowskii Zal.', 211
 Brian, P. W., Curtis, P. J. & Hemming, H. G.
 'A substance causing abnormal de-
 velopment of fungal hyphae produced by
 Penicillium janczewskii Zal. I. Biological
 assay, production and isolation of
 "curling factor", 173
 Bromus inermis, 165
 Buxus sempervirens (box), 260

 Cactus, 43
 Cadman, C. H., 'Raspberry pathology in
 Scotland', 189
 Camellia, 142
 Candida Berkh., 100
 Canter, H. M., 'Studies in British Chytrids. I.
 Dangeardia mammillata Schröder', 128
 Caragana arborescens, 65
 Carex, 193, 254
 muricata, 254
 siccata, 254
 Carpinus (hornbeam), 195, 207
 Castanea sativa (vesca) (sweet chestnut), 151,
 220
 Centaurea Scabiosa, 13
 Cercospora depazeoides (Desm.) Sacc., 251
 Chaetomium Kunze ex Fr., 70
 cancroideum Tschudy, 70
 dolichotrichum Ames, 70
 fiscicolum Petrak, 70
 globosum Kunze, 5, 70
 hispidum Fr., 70
 microcephalum Ames, 70
 ochraceum Tschudy, 70
 pachypodioides Ames, 70
 subterraneum Swift & Povah, 70
 tetrasperum S. J. Hughes, n.sp., 70, 71, 72
 tortuosum Garbowski, 70
 Chytridium A. Br., 68, 134, 135
Chytridium Lecythii (Ingold) n.comb., by E. K.
 Goldie Smith, 68
 *Chytridium Lecythii (Ingold) Goldie Smith,
 n.comb., 68, 69
 Chytriomycetes Karling, 137
 **nodulatus* Haskins, n.sp., 137, 139
 Citrus, 165
 Cladium, 193
 Cladochytrium Nowakowski, 135
 hyalinum Berdan, 135
 Cladosporium Link ex Fr., 163, 232
 herbarum Link ex Fr., 163
 *Clavaria Botrytis (Pers.) Fr., var. *alba*
 A. A. Pearson, n.var., 209
 fennica Karst., 209
 Kunzei Fr., 97
 versatilis Quél., 209
 Clisporium Fr., 40
 Cochlonema dolichospora Drechs., 170
 verrucosum Drechs., 170
 Colletotrichum linicola Pethyb. & Laff., 221
 Collybia butyracea (Bull.) Fr., 59
 maculata (Alb. & Schw.) Fr., 55
 Colpoma quercinum (Fr.) Wallr., 234, 235,
 237, 240
 Comatricha aequalis Peck, 260
 nigra Schroet., 260
 var. *aequalis* Sturgis, 260
 Coniothyrium Sacc., 155, 156
 piricola Potebnia, 155, 157
 pirina Sheldon, 156, 157
 pirinum (Sacc.) Sheldon, 155, 157
 tirolense Bubák, 155, 157
 Conway, E., 'Exhibit of Japanese and British
 agars', 189
 Cook, W. R. Ivimey, 'The Phycomycete
 Flora of South Wales', 95
 Coprinus (Pers. ex Fr.) S. F. Gray, 53, 54
 atramentarius (Bull.) Fr., 54
 comatus (Fl. Dan.) Fr., 54
 lagopus Fr., 54
 micaceus (Bull.) Fr., 54, 55
 niveus (Pers.) Fr., 54
 papillatus (Batsch) Fr., 54
 plicatilis (Curt.) Fr., 54
 radiatus (Bolt.) Fr., 54
 Cordana pauciseptata Preuss, 97
 Cordyceps capitata (Holmsk.) Ces. & de Not.,
 97
 ophioglossoides (Ehrh.) Fr., 97
 Corticium Solani (Prill. & Delacr.) Bourd. &
 Galz., 255, 256
 *Cortinarius ammophilus A. A. Pearson,
 n.sp., 198
 balaustinus Fr., 97
 bolaris (Pers.) Fr., 97, 197

- brunneus (Pers.) Fr., 197
 damascenus (Pers.) Fr., 97
 delibutus Fr., 97
 evernius Fr., 97
 holophaeus Lange, 197
 limonius Quél. non Fr., 197
 melleopallens Fr., 97
 penicillatus Fr., 97
 pseudobolaris R. Maire, 197
 rubicundulus (Rea) A. A. Pearson, n.comb., 197
 saniosus Fr., 97
 Corylus (hazel), 97, 197, 207
 Coryne sarcoides (Jacq.) Tul., 152
 Covillea glutinosa, 43
 Crambe (seakale), 256
 Crepis, 254
 Cribraria aurantiaca Schräd., 260
 microcarpa (Schräd.) Pers., 261
 vulgaris Schräd., 260
 var. aurantiaca Pers., 260
 Crocus sativus (saffron), 114
 Croxall, H. E., 'The twentieth annual plant pathology field day', 263
 Curtis, P. J., *see* Brian, P. W.
 Cystopage lateralis Drechs., 170
 Cystopteris montana, 144
 Cytisus, 64
 Laburnum, 65
 scoparius, 64-7

 Dactylella bembicoides Drechs., 170
 Dactylis glomerata (cock's foot), 164, 196
 Dactylium dendroides Fr., subsp. leptosporum Sacc., 158
 Daily census of *Alternaria* spores caught from the atmosphere at Cardiff in 1942 and 1943, by H. A. Hyde and D. A. Williams, 78
 Daldinia Ces. & de Not., 43, 47
 concentrica Ces. & de Not., 43, 44, 50, 51, 97
 Dandelion yellow mosaic virus, 252
 Dangeardia Schröder, 133
 mammillata Schröder, 128-33
 Daucus Carota (carrot), 255
 Davies, E. A. V., 'Pythiaceae', 95
 Davies, W., 'Mucorales', 95
 Deconica W. G. Smith, 142
 Dematium pullulans de Bary, 87
 Dendrophoma Sacc., 38
 faginea Ferraris, 38
 Dennis, R. W. G., 'Notes on some British fungi ascribed to *Phoma* and related genera', 11
 Dennis, R. W. G. & E. M. Wakefield, 'New or interesting British fungi', 141
 Dianthus, 163
 Caryophyllus (carnation), 165
 Diatrype Stigma (Fr.) Fr., 97
 Didymium anomalum Sturgis, 260
 eximium Peck, 260
 nigripes Fr., 260
 Sturgisii Hagelstein, 260
 xanthopus Fr., 260
 Digraphis arundinacea, 145
 Diplodina Westend., 21, 26, 39, 40
 Gallii (Niessl) Sacc., 13, 37
 Grossulariae Sacc. & Bri., 13
 Lycopersici Hollos, 13, 15, 23, 25, 26, 27, 39
 Sonchi P. Henn., 13, 26
 Diplophlyctis Schroet., 135
 Dovaston, H. F., 'The ascigerous stage of a species of *Epicoccum*', 189
 Dryopteris, 143, 144
 Felix-mas, 144
 Duddington, C. L., 'Predacious fungi in Britain', 170

 Ecology of the larger fungi, by J. Grainger, 52
 Elaeomyxa Myazakiensis (Emoto) Hagelstein, 260
 Elaphomyces Nees ex Fr., 97
 Ellis, E. A., '*Symphosira* Preuss and two British Discomycetes with *Symphosira*-like conidial organs', 189
 Endomycopsis albicans (Vuill.) Dekker, 174
 Endothia parasitica (Murr.) Anderson & Anderson, 43
 Enderthenema Berkeleyanum Rost., var. syn-carpon (Sturg.) G. Lister, 260
 melanospermum Macbr. & Martin, 260
 papillatum Rost., 260
 Entoloma porphyrophaeum Fr., 60
 Entophlyctis A. Fischer, 138
 *aurea Haskins, n.sp., 138, 139
 Epicoccum Link ex Wallr., 189
 Erodium cicutarium, 153
 Erysiphe Cichoracearum DC., 251
 communis (Wallr.) Fr., 250
 Polygoni DC., 250
 Escallonia, 141
 Escherichia coli (Migula) Castell. & Chalm., 174
 Eudorina elegans, 128, 133
 Euphorbia, 65, 66
 Cyparissias, 64-6, 167
 virgata, 64
 Eurotium Link ex Fr., 147
 insigne G. Winter, 146, 147
 Eurya acuminata, 143
 Exobasidium Woron., 142
 Brevieri Boudier, 143
 Camelliae Shirai, 142, 143
 Euryae Syd. & Butl., 143
 Exosporium depazeoides Desm., 251

 Fagus (beech), 195, 197, 204, 209, 220, 247, 251
 Festuca elatior, 160
 Flammula dactylidicola Lange, 196
 flava (Bres.) Lange, 196
 rubicundula Rea, 197
 Foister, C. E., *see* Boyd, A. E. W.
 Fomes annosus (Fr.) Cooke, 57, 60
 Foray Report, 1945, 97
 Fraxinus excelsior (ash), 13, 30, 75
 Funkia, 92
 Fusarium Link ex Fr., 122, 164, 263
 caeruleum (Lib.) Sacc., 11
 citriforme Jamalainien, 164, 165
 culmorum (W. G. Smith) Sacc., 116, 221
 Lini Bolley, 221-4, 226, 228, 229
 oxyosporum Fr., f. Lini Bolley, 221
 Poae (Peck) Wollenw. ap. Lewis, 164

- Galera appendiculata Lange & Kühner, 196,
197
hypnorum (Schrank) Fr., 58
ravida Fr., 197
Galium Aparine (goosegrass), 13, 37
Mollugo, 37
Garrett, S. D., 'A study of violet root rot.
Factors affecting production and growth
of mycelial strands in *Helicobasidium
purpureum* Pat., 114
'A multiple-point inoculating needle for
agar plates', 171
Geastrum fimbriatum Fr., 97
Genista anglica, 64-7
Geum, 153, 154
album, 154
coccineum, 155
macrophyllum, 155
rivale, 155
urbanum, 155
Gladiolus, 150
Glasscock, H. H. & Ware, W. M., '*Uromyces
striatus* Schroet. on *Medicago lupulina* L.
and other host plants in Britain', 107
Gliocladium Corda, 147
fimbriatum Gilman & Abbott, 211
macropodium Marshal, 147
penicilloides Corda, 146, 147
Gloeosporium filicinum Rostr., 143
Glomerella piperata (Stonem.) Spauld. & v.
Schrenk, 87
Godetia, 163
Gonatobotryum, fuscum Sacc., 97
Grainger, J., 'Ecology of the larger fungi', 52
Gray, E., 'Problems in plant pathology in the
North of Scotland', 189

Haematomyces fagineus Peck, 152
Harposporium anguillulae Lohde, 170
Haskins, R. H., 'New chytridiaceous fungi
from Cambridge', 135
Hedera Helix, 13
Helianthemum, 93
Chamaecistus, 93
montanum, 93
vulgare (rock rose), 93
Helicobasidium purpureum Pat., 114-19,
122-6
Helminthosporium Link ex Fr., 159, 161, 185
dematioideum Bubák & Wróblewski, 160
dictyoides Drechs., 159
erythrospilum Drechs., 162
Leersii Atk., 161
siccans Drechs., 160
stenacrum Drechs., 161
triseptatum Drechs., 160, 161
tritici-repentis Diedicke ex Drechs., 159
velutinum Link ex Fr., 189
Helosciadium nodiflorum, 153
Hemming, H. G., *see* Brian, P. W.
Heracleum Sphondylium, 13, 32
Herpobasidium filicinum (Rostr.) Lind, 143
Heterosporium Klotzsch ex Cooke, 90-2
Allii Ellis & Martin, 90, 92
var. Allii-Porri Sacc. & Br., 90, 92
var. Bomareae Pat., 92
var. cepivorum Nicolas & Aggéry, 90, 91,
92
var. Funkiae Massal., 92
var. Polygonati Oud., 92
Allii-Cepae Jørstad, 90
Allii-Cepae Ranojević, 90, 91, 92
Ornithogali Klotzsch, 90
var. Allii-Porri Sacc. & Br., 90
Hieracium, 254
Holcus, 60
lanatus, 160
Hordeum (barley), 83, 165
Hormodendrum Bon., 79, 80, 88, 163
cladosporioides (Fres.) Sacc., 163
Howard, H. J. Review: '*The Mycetozoa of
North America, based upon the specimens in the
Herbarium of the New York Botanical
Garden*', by R. Hagelstein, 259
Hughes, S. J., 'An undescribed species of
Chaetomium, with four-spores asci', 70
'Some specimens called *Helminthosporium
velutinum*', 189
Humaria granulata (Bull.) Qué., 56
Hyacinthus (hyacinth), 252
Hyde, H. A. & Williams, D. A., 'A daily
census of *Alternaria* spores caught from the
atmosphere at Cardiff in 1942 and 1943',
78
Hyde, H. A., *see* Jenkins, E. A., Williams, D. A.
Hydnum cirrhatum (Pers.) Fr., 208
graveolens (Delast.) Fr., 97
scrobiculatum Fr., 97
Hygrophorus Fr., 53, 54
calyptraeformis Berk., 54
ceraceus (Wulf.) Fr., 54
chlorophanus Fr., 54, 55, 58
coccineus (Schaeff.) Fr., 54
conicus (Scop.) Fr., 54
discoideus (Pers.) Fr., 195
discoideus Qué. non Fr., 195
eburneus (Bull.) Fr., 54
laetus (Pers.) Fr., 59, 98
leucophaeus (Scop.) Fr., 195
mesotephrus B. & Br., 195
metapodium Fr., 54
miniatus Fr., 54
nigrescens Qué., 54
niveus Cooke, 54
olivaceoalbus Fr., 98
pratensis (Pers.) Fr., 54
psittacinus (Schaeff.) Fr., 54
puniceus Fr., 54
subradiatus (Schum.) Fr., 54
virginus (Wulf.) Fr., 54
Hymenobolus parasitica Zukal, 261
Hypholoma cyanescens R. Maire, 165
fasciculare (Huds.) Fr., 55
Hypomyces aureo-nitens Tul., 147
Hypoxylon fuscum (Pers.) Fr., 44, 97

Ingold, C. T., 'Size and form in agarics', 108
'Spore discharge in *Daldinia concentrica*', 43
Inocybe dulcamara (Alb. & Schw.) Fr., 200
euthelus B. & Br., 199
globocystis Vel., 97
griseo-lilacina Lange, 97, 201

- hystrich Fr., 97
 lacera Fr., 201
 Langei Heim, 199
 lanuginosa (Bull.) Fr., 199, 200
 longicystis G. F. Atkinson, 200
 obscura (Pers.) Fr., 201
 ovatocystis Kühner & Boursier, 199
 scrotina Peck, 200
 striata Bres., 198
 tomentosa (Jungb.) Quél., 199
 Iris, 150
 Irpex pachyodon (Pers.) Quél., 209
 Isoachlya anisospora (de Bary) Coker var.
 indica Saksena & Bhargava, 101, 105, 106
 Jenkins, E. A. & Hyde, H. A., 'The collection
 of fungus models in the Department of
 Botany, National Museum of Wales', 95
 Jones, E. S. L., 'Chytridiales', 95
 Juglans mandschurica, 157
 Kleistobolus pusillus Lipp., 261
 Krombholzia scabra Singer var. chromapes
 Frost, 208
 Laburnum, 64
 Laccaria laccata (Scop.) B. & Br., 55, 58
 Lactarius Fr., 53, 54
 camphoratus (Bull.) Fr., 54
 ichoratus (Batsch) Fr., 54
 piperatus (Scop.) Fr., 54
 pyrogalus (Bull.) Fr., 54, 58
 quietus Fr., 54
 rufus (Scop.) Fr., 54
 subdulcis (Pers.) Fr., 54, 55, 58
 'subtomentosus', 54
 subumbonatus Lindgr., 54
 terminosus (Schaeff.) Fr., 54
 turpis (Weinm.) Fr., 54, 60
 Lactuca canadensis, 254
 muralis, 254
 perennis, 254
 sativa (lettuce), 163, 252, 253, 254, 256
 Scariola, 254
 Lagenidium Schenk, 135
 Lamproderma atrosporum Meylan, 260
 var. anglicum G. Lister & Howard, 260
 var. debile G. Lister & Howard, 260
 cribrarioides R.E.Fr., 260
 Gulielmae Meylan, 260
 Lapsana communis, 13, 32, 254
 Larix (larch), 148, 200
 Lecythium hyalinum, 68, 69
 Lepidoderma Carestianum Rost., 260
 var. granuliferum R.E.Fr., 260
 Chaillatii Rost., 260
 Lepiota felina (Pers.) Fr., 192
 pseudo-felina Lange, 192
 Leptolegnia caudata de Bary, 105
 Leptospora caudata Fuckel, 148
 spermoides (Hoffm.) Fuckel, 148
 Licipenicillium insigne Brefeld, 146
 Lillium lanceolatum, 90
 Lilliputia Boud. & Pat., 147
 Gaillardii Boud. & Pat., 147
 *insigne (Winter) Wakef., n.comb., 145
 Linaria maroccana, 163
 Linum usitatissimum (flax), 13, 21, 221, 256
 Loborhiza Metzneri A. Hanson, 133
 Lolium, 160
 Loniceria Periclymenum (honeysuckle), 13, 21
 Lophocolea bidentata, 149
 cuspidata, 149
 Lycoperdon giganteum (Batsch) Pers., 52
 pyriforme (Schaeff.) Pers., 55, 58
 saccatum (Vahl) Fr., 98
 Lycopersicum esculentum (tomato), 13, 15,
 16, 38, 40, 56, 256
 Lysurus Fr., 141
 McGowan, J. C., 'A substance causing
 abnormal development of fungal hyphae
 produced by *Penicillium janczewskii* Zall.
 II. Preliminary notes on the chemical
 and physical properties of "curling
 factor"', 188
 McLean, R. C., 'The aerial micro-organisms',
 95
 Macbrideola scintillans Gilbert, 260
 Macdonald, J. A., '*Uromyces Genistae-tinctoriae*
 (Pers.) Wint., in Scotland', 64
 Macfarlane, I., see Robertson, N.
 Macrophoma (Sacc.) Berl. & Vogl., 12
 Mappia ovata, 86-8
 Marasmius Hudsonii (Pers.) Fr., 108
 oreadoides (Passer.) Fr., 194, 195
 Mason, E. W., 'The name *Helminthosporium*
 velutinum', 189
 Matthiola, 256
 Medicago, 167
 arabica, 168, 169
 falcata, 165
 lupulina (trefoil), 167, 169
 maculata, 168
 sativa (lucerne), 114, 165, 167
 Melilotus, 165
 Memnoniella echinata (Riv.) Galloway, 5
 Mercurialis perennis (dog's mercury), 114
 Meria coniospora Drechs., 170
 Mespilus germanica (medlar), 97
 Microsphaera Lév., 219
 alphitoides Griff. & Maubl. (oak mildew),
 98, 219, 220
 densissima (Schw.) Cooke & Peck, 220
 quercina (Schw.) Burr., 220
 Molinia, 193
 caerulea, 193
 Montia fontana, 145
 Moore, M. H. Review: '*Fungicides and their*
 action', by J. G. Horsfall, 98
 Moore, W. C., 'New and interesting plant
 diseases', 90, 250
 Morchella Dill. ex Fr., 53
 esculenta (L.) Pers., 53, 55
 Morgan, E., '*Saprolegniales*', 95
 Mucor Mich. ex Fr., 186
 Multiple point inoculating needle for agar
 plates, by S. D. Garratt, 171
 Mycena (Fr.) S. F. Gray, 194
 arcangeliana Bres., 194
 var. Oortiana Kühner, 193
 erubescens v. Höhn., 192, 193

- Mycena* (cont.)
 fellea Lange, 192, 193
 galopus (Pers.) Fr., 192
 metata Fr., 194
 quisquiliaris (Josser.) Kühner, 193
 vitrea Fr., var. tenella, 194
Mycobacterium tuberculosis Lehm. & Neum., 211
Mycosphaerella Johanson, 88
Myriangium, by T. Petch, 74
Myriangium asterinosporum (Ell. & Everh.) Miller, 74-7
 Berkeleyi Mont., 75
 Curtisii Mont. & Berk., 74-6
 Duriaci Mont. & Berk., 74-6
Myzus ascalonicus, 253
 ornatus, 253
 persicae, 253
 pseudosolani, 253
- Natural occurrence of sprouting of the ascospores of *Sphaerulina Mappiae* (Petch) n.comb., in Ceylon, by T. E. T. Bond, 86
Naucoria flava Bres., 196
 Myosotis Fr., 142
Nectria cinnabarina (Tode ex Fr.) Fr., 57
Nectriella Lophocoleae Massal., 148
 New and interesting plant diseases, by W. C. Moore, 90, 250
 New chytridiaceous fungi from Cambridge, by R. H. Haskins, 135
 New or interesting British fungi, by R. W. G. Dennis and E. M. Wakefield, 141
Nicotiana, 163
 Noble, M., 'A brief history of early mycological work in Scotland', 189
 Note on the occurrence of species of *Ooidendron* Robak in Britain, by G. Smith, 232
 Notes on some British fungi ascribed to *Phoma* and related genera, by R. W. G. Dennis, 11
 Notes on the die-back of oak caused by *Colpoma quercinum* (Fr.) Wallr., by E. S. Twyman, 234
 Notice: List of Common British Plant Diseases, 100
Nowakowskiella Schroet., 135
 hemisphaerospora Shanor, 135
- Oak mildew, 98, 219
 Observations on wilt disease of flax, by I. M. Wilson, 221
 Occurrence of perithecia of the oak mildew in Britain, by N. Robertson and I. Macfarlane, 219
Ooidendron Robak, 163, 232, 233
 fuscum Robak, 163, 232
 nigrum Robak, 163, 232
 rhodogenum Robak, 163, 232
Oidium (Link) Sacc., 163, 250, 251
 aureum Link, 98
 erysiphioidea Fr., 250
 Valerianellae Fuckel, 250
Olpidopsis Cornu, 135
Olpidium (A. Br.) Rabenh., 135
Omphalia (Fr.) S. F. Gray, 194
 candida Bres., 194
 integrella Fr., 194
 Mairei Gilbert, 194
 quisquiliaris Josserand, 193
 Oogenesis and fertilization in *Isoachlya anisospora* var. *indica*, by K. S. Bhargava, 101
Ophiobolus graminis (Sacc.) Sacc., 116
Ophionectria cerea (Berk. & Curt.) Ell. & Everh., 97
Orcadella Wingate, 261
 parasitica (Zukal) Hagelstein, 261
- Paecilomyces* Bainier, 5
 Varioti Bainier, 5
Panaeolus phalaenarum Fr., 55
Pandorina Morum, 128
Panus torulosus (Pers.) Fr., 60
Pastinaca sativa (parsnip), 255
Paxillus involutus (Batsch) Fr., 55, 58
 Pearson, A. A., 'New records and observations. III', 191
 Review: 'Flora Agaricina Danica', by J. E. Lange, 99
Pediculopsis graminum, 165
Penicillium Link ex Fr., 5, 78, 211
 brevi-compactum Diereks, 5
 chrysogenum Thom., 8
 insigne Bainier, 146
 Janczewskii Zal., 173-6, 179-82, 186, 188
 Jenseni Zal., 211, 212, 213
 notatum Westl., 8
 obscurum Biourge, 211, 214
 Terlikowskii Zal., 211, 212, 214, 215, 216, 217
Perichaena corticalis (Batsch) Rost., 259
Peronospora Erodii Fuckel, 153
 Gei Sydow, 153
 Perrott, P. E., 'Monoblepharidales', 95
 Petch, T., 'Myriangium', 74
Phalaris arundinacea, 145
Phallus impudicus (Linn.) Pers., 55, 58
Phleum, 165
Pholiota Fr., 242
 adiposa Fr., 242, 244, 246, 248, 249
 heteroclita Fr., 242, 245, 247
 mutabilis (Schaeff.) Fr., 242, 245, 247, 249
 spectabilis Fr., 242, 243, 245, 247, 248, 249
 squarrosa (Müll.) Fr., 242, 246-9
Phoma Fr., 11, 12, 13, 14, 16, 21, 26, 31, 34, 36, 39, 40
 Aceris-negundinis Arcangel., 13, 29
 acuta Fuckel, 13, 33, 36
 alternariacearum Brooks & Searle, 12, 13, 14, 38
 Bismarckii Kidd & Beaumont, 29
 complanata Desm., 13, 32
 destructiva Plowr. cm. Jameson, 27, 39, 40
 ellipticum Peck, 39
 eupyrena Sacc., 11, 12, 13, 31
 foveata Foister, 11, 13, 14, 16, 17, 21, 37
 glomerata (Corda) Wollenw. & Hochapfel, 39
 herbarum Westend., 13, 26, 30, 40
 f. Galiorum Sacc., 37
 herbarum Westend., f. Solidaginis Sacc., 13, 36
 hibernica Grimes et al., 13, 33, 34, 40

- Kalkhoffi Bubák, 26
 Lampsanae Karst., 13, 33
 Libertianae Speg. & Roum., 39
 linella Desm., 26
 lingam (Fr.) Desm., 13, 15, 26, 38
 Lini Passer., 26
 linicola Marchal & Verplancke, 26
 Mori Mont., 39
 nebulosa (Pers.) Mont., 11, 13, 35, 39
 oleracea Sacc., 11, 13, 34, 36, 40
 var. *Scrophulariae* Sacc., 36
 prunicola Schwein., 39
 samararum Desm., 29
 sanguinolenta Grove, 13, 33
 sarmentella Sacc., 39
 seriata (Pers.) Wollenw. & Hochapfel, 40
 Solani Cooke & Harkn., 11
 solanicola Prill. & Delacr., 11, 13, 22, 25, 26
 Solidaginis Cooke, 36
 striaeformis Dur. & Mont., 29, 39
 terrestris Hansen, 22
 tuberosa Melhus, Rosenbaum & Schultz, 11, 13, 26
 Urticae Sch. & Sacc., 13, 24, 35
 vincicola Gonz, 26
 Phomopsis Sacc., 12, 26
 Phyllosticta Pers. ex Desm., 12, 21, 39, 156, 157, 250
 Briardi Sacc., 157
 Grossulariae Sacc., 13, 30
 hedericola Dur. & Mont., 13, 28, 40
 helianthemicola Allesch., 93
 Lonicerae Westend., 13, 26
 Mali Prill. & Delacr., 155, 156, 157
 pirina Sacc., 156, 157
 Rabiei (Pass.) Trott., 39
 Solidaginis Bres., 37
 tirolensis Bubák, 157
 Physarum Pers. em. Rost., 260
 Bilgramii Hagelstein, 259
 carneum G. Lister & Sturgis, 260
 columbinum Sturgis, 259
 connatum Lister, 260
 lilacinum Fr., 259
 lilacinum Sturgis & Bilgram, 259
 Listeri Macbr., 259
 luteo-album Macbr., 259
 luteo-album Schumacher, 259
 notabile Macbr., 260
 pezizoideum Pav. & Lag., 260
 Wingatense Macbr., 259
 Phytophthora de Bary, 261
 Fragariae Hickman, 263
 Picea (spruce), 195
 Pinus (pine), 192, 199
 Pisum sativum (pea), 165
 Plasmodiophora Brassicae Woron., 116
 Plasmopara Erodii (Fuckel) D. Sacc., 153
 Plowrightia ribesia (Pers. ex Fr.) Sacc., 87
 Pluteus cervinus (Schaeff.) Fr., 57
 Godeyi Gillet, 196
 semibulbosus (Lasch) Fr., 196
 Poa, 165
 pratensis, 164
 Polygonatum, 92
 Polypodium, 143, 144
 Phegopteris, 144
 Polyporus betulinus (Bull.) Fr., 60
 Schweinitzii Fr., 97
 Polyspora Lini Laff., 221, 223, 224, 226, 228, 230
 Populus (poplar), 208
 Predacious fungi in Britain, by C. L. Duddington, 170
 Prenanthes, 254
 President: Dr Ramsbottom, 96
 Mr G. Smith, 95
 Presidential Address: Mycology and the war, by G. Smith, 1
 Proceedings, 95, 189
 Production of gliotoxin by *Penicillium Terlikowskii* Zal., by P. W. Brian, 211
 Protascus subuliformis Zopf, 170
 Protomyces Unger, 153
 Helosciadii Phill., 153
 inundatus Dangeard, 152, 153
 macrosporus Unger, 152
 Prunus persica (peach), 165
 *Psalliota arenicola Wakef. & Pearson, n.sp., 205
 arvensis (Schaeff.) Fr., 204
 campestris (L.) Fr., 44, 54, 56, 61
 *litoralis Wakef. & Pearson, n.sp., 205
 meleagris Schaeff., 203
 silvatica Schaeff., 203
 subperonata Lange, 202
 vaporaria (Vitt.) Schaeff., 202
 villatica (Brond.) Magn., 204
 xanthoderma Genev., 203
 *var. grisea A.A. Pearson, n. var., 203, 204
 var. lepiotoides Maire, 203
 var. obscurata Maire, 203
 Pseudonectria Seaver, 149
 Pseudoperonospora Erodii (Fuckel) G. W. Wilson, 153
 Pseudosphaeria v. Höhn., 147
 Psilocybe (Fr.) Quel., 142, 165
 caerulescens Murr., 142
 coprophila (Bull.) Fr., 58
 *cyanescens Wakef., n.sp., 141, 165
 semilanceata Fr., 55, 58
 Puccinia Chondrillae Corda, 254
 commutata Syd., 144
 extensicola Plowr., var. hieraciata (Schw.) Arth., 255
 graminis-tritici, 66
 Lactucarum Syd., 254
 Opizii Arth. non Bubák, 255
 Opizii Bubák, 254
 patruelis Arth., 255
 Porri (Sow.) Wint., 91
 prenanthis (Pers.) Lindr., 254, 255
 'striatum', 168
 Valerianae Auctt. non Carest., 144
 Valerianae Carest., 144
 Pyrus, 157
 Malus (apple), 15, 16, 40
 Pythiomorpha Petersen, 261
 Fischeriana Höhnk, 261
 Quercus (Oak), 195, 199, 208, 209, 219, 234, 251

- Raphanus sativus (radish), 255, 256
 Receipts and Payments, 96
 Reviews: 'Die Hefesammlung des "Centraalbureau voor Schimmelcultures"'. Beiträge zu einer Monographie der Hefarten. II. Teil. Die anaskorosporogenen Hefen. Zweite Hälfte.' (H. A. Diddens & J. Lodder), by B. Barnes, 100
 'Flora Agaricina Danica' (J. E. Lange), by A. A. Pearson, 99
 'Fungicides and their action' (J. G. Horsfall), by M. H. Moore, 98
 'The aquatic Oomycetes of Wisconsin. Part I' (F. T. Wolf), by E. M. Blackwell, 261
 'The Mycetozoa of North America, based upon the specimens in the Herbarium of the New York Botanical Garden' (R. Hagelstein), by H. J. Howard, 259
 Rhizidiomyces Zopf, 135
 Hansonii Karling, 135
 Rhizidium ramosum Sparrow, 135
 Rhizoctonia DC., 114
 crocorum Fr., 114, 115
 medicaginis DC., 114
 Solani Kühn, 116, 255
 violacea Tul., 114
 Rhizophidium Schenk, 134
 Eudorinae O. Hood, 133, 134
 Lecythii Ingold, 68, 69
 Rhizophlyctis Petersenii Sparrow, 138
 Rhodophyllus Quél., 99
 Rhytisma salicinum (Pers.) Fr., 149, 150
 symmetricum J. Müller, 149
 Ribes Grossulariae (gooseberry), 13, 37
 Robertson, N. & Macfarlane, I., 'The occurrence of perithecia of the oak mildew in Britain', 219
 de Rousset-Hall, O., 'Exhibit of agarics preserved by freeze drying', 189
 Rubus, 193
 Idaeus (raspberry), 189
 Russula Pers. ex S. F. Gray, 53, 54
 adusta (Pers.) Fr., 54, 60
 atropurpurea (Krombh.) Maire, 54, 55, 58
 cutefracta Cooke, 54
 cyanoxantha (Schaeff.) Fr., 54, 55, 58
 elephantina Fr., 54
 emetica (Schaeff.) Fr., 54, 55, 58, 59
 fellea Fr., 59
 fragilis (Pers.) Fr., 54
 gracillima Schaeff., 97
 graminicola (Secr.) Quél., 54
 lactea (Pers.) Fr., 54
 luteo-viridans Martin, 195
 luteotacta Rea, 54
 nigricans (Bull.) Fr., 54, 55, 58, 59, 60
 ochroleuca (Pers.) Fr., 54, 55, 58
 olivacea (Schaeff.) Fr., 54
 urens Romell, 195
 Velenovskyi Melzer & Zvara, 54
 venosa Velen., 54
 xerampelina (Schaeff.) Fr., 59
 Salix, 150
 repens, 198
 Salmonella typhi Warren & Scott, 174, 175
 Sambucus (elder), 193, 251
 Sampson, K., 'Notes on the biology of British smuts', 189
 Saprolegnia Nees ex Pringsh., 135
 dioica de Bary, 106
 ferax (Gruith.) Thuret, 106
 mixta de Bary, 106
 Sarothamnus scoparius, 64
 Schizanthus, 251
 Scilla, 252
 Scleroderma aurantium Pers., 55, 58, 60
 Sclerotinia Fuckel, 150
 bulborum (Wakk.) Rehm, 252
 *Draytoni Buddin & Wakef., n.sp., 150, 151
 fructigena Adher. & Ruhl., 3
 gladioli Drayton, 150
 hirtella Boud., 151
 Mespili Schell., 97
 sclerotium (Lib.) de Bary, 186
 tuberosa (Fr.) Fuckel, 250, 251
 Sclerotium crocorum Pers., 114
 Scrophularia, 36
 nodosa (figwort), 13, 35
 Secale cereale (rye), 165
 Senecio, 11, 26
 Jacobaea (ragwort), 13, 21
 Septocylindrium Aspidii Bres., 158
 Septogloeum Mappiae Petch, 88, 89
 Septoria Sacc., 29, 93
 Chamaecisti Vesterg., 93
 Helianthemii Frag., 93
 Helianthemii (Vesterg.) Allesch., 93
 Sistotrema pachyodon (Pers.) Fr., 209
 Sisymbrium officinale, 13, 32
 Sium erectum, 153
 Size and form in agarics, by C. T. Ingold, 108
 Smith, E. K. Goldie, 'Chytridium Lecythii (Ingold) n.comb.', 68
 Smith, G., 'Presidential Address: Mycology and the War', 1
 'Exhibit of moulds and mould products', 95
 'Note on the occurrence of species of Oidiendendron Robák in Britain', 232
 Solanum tuberosum (potato), 13, 14, 16, 40, 117, 255, 256
 Solidago Virgaurea (goldenrod), 13, 36
 Sonchus, 254
 asper, 26
 oleraceus (sowthistle), 13, 21
 Sordaria caudata (Curr.) Sacc., 147
 Sorosporium Montiae Rostr., 145
 Sparassia laminosa Fr., 97
 Sphaerella Ces. & de Not., 87, 88
 Mappiae Petch, 86, 88
 Sphaeria Haller ex Fr., 40
 caudata Currey, 147
 falcispora Broome, 148
 Sphaeropsis Sacc., 158
 malorum Peck, 122, 158
 Sphaerulina Sacc., 87, 88, 89
 intermixta (B. & Br.) Sacc., 87
 Mappiae (Petch) T. E. T. Bond, n.comb., 86, 88, 89
 myrtilina Sacc. & Fautr., 147
 Taxi Massee, 88

- Spore discharge in *Daldinia concentrica*, by C. T. Ingold, 43
- Sporotrichum Link ex Fr., 164
- Poa Peck, 164
- Stachybotrys atra Corda, 5
- Staphylococcus aureus Rosenbach, 174, 175
- Stemonitis axifera (Bull.) Macbr., 260
- ferruginea Ehrenb., 260
- fusca Roth., 260
- trechispora (Torr.) Macbr., 260
- Stereum Karstenii Bres., 60
- purpureum (Pers.) Fr., 57
- rugosum (Pers.) Fr., 57
- Strasseria carpophila Bres. & Sacc., 158
- Stropharia merdaria Fr., 55
- rugoso-annulata Farlow, 201, 202
- semiglobata (Batsch) Fr., 55, 56, 58
- stercoraria Fr., 55, 56
- Studies on British Chytrids. I. *Dangeardia mammillata* Schröder, by H. M. Canter, 128
- Study of violet root rot. Factors affecting production and growth of mycelial strands in *Helicobasidium purpureum* Pat., by S. D. Garrett, 114
- Stylopaga Drechsler, 170
- hadra Drechsler, 170
- haploe Drechsler, 170
- lepte Drechsler, 170
- Substance causing abnormal development of fungal hyphae produced by *Penicillium Janczewskii* Zal. I. Biological assay, production and isolation of 'curling factor', by P. W. Brian, P. J. Curtis and H. G. Hemming, 173
- Substance causing abnormal development of fungal hyphae produced by *Penicillium Janczewskii* Zal. II. Preliminary notes on the chemical and physical properties of 'curling factor', by J. C. McGowan, 188
- Symphosira Preuss, 189
- Symphytum (comfrey), 194
- Taphridium Lagerh. & Juel, 153
- Taraxacum (dandelion), 252
- Thea japonica, 142
- Thraustotheca clavata (de Bary) Humphrey, 103, 105
- Tilletia Menieri Har. & Pat., 145
- Tolyposporium Montiae Rostr., 145
- Tortrix viridana, 237
- Torula Pers. ex Fr., 163
- Torulopsis utilis (Henneb.) Lodder, 3
- var. major Thaysen & Morris, 3
- var. thermophila Thaysen, 3
- Trametes mollis (Sommerf.) Fr., 57
- Trichamphora Jungh., 260
- pezizoidea Jungh., 260
- Trichoderma viride Pers. ex Fr., 5, 175, 211, 213, 214, 216
- *Tricholoma ionides (Bull.) Fr., var. obscurissima A. A. Pearson, n.var., 192
- melaleucum (Pers.) Fr., 192
- Trichosporon Behrend, 100
- Trifolium, 169
- agrarium, 169
- arvense, 169
- minus, 168, 169
- pratense (red clover), 114, 165
- procumbens, 168, 169
- Triticum (wheat), 83, 165
- Tritona uvaria, 90
- Tuberculina Sacc., 114
- Twentieth annual plant pathology field day, by H. E. Croxall, 263
- Twyman, E. S., 'Notes on the die-back of oak caused by *Colpoma quercinum* (Fr.) Wallr.', 234
- Ulex europaeus, 64-7
- Ulmus (elm), 192
- Undescribed species of *Chaetomium* with four-spored asci, by S. J. Hughes, 70
- Uromyces Link, 168
- Genistae-tinctoriae (Pers.) Wint., 64, 66, 67
- *f. anglicae J. A. Macdonald, n.f., 67
- *f. scoparii J. A. Macdonald, n.f., 67
- *f. Ulicis J. A. Macdonald, n.f., 67
- Jaapianus Kleb., 168, 169
- striatus Schroet., 167, 168, 169
- Uromyces Genistae-tinctoriae (Pers.) Wint., in Scotland, by J. A. Macdonald, 64
- Uromyces striatus Schroet. on *Medicago lupulina* L., and other host plants in Britain, by H. H. Glasscock & W. M. Ware, 167
- Urtica dioica (nettle), 13, 32, 34, 35, 114
- Ustilago echinata Schroet., 145
- longissima (Schlecht.) Meyer, 145
- striiformis (Westend.) Niessl, 145
- Ustilina Tul., 237
- Vaccinium Myrtillus, 147
- Valeriana celtica, 144
- officinalis, 144, 250
- Valerianella, 250
- carinata, 250
- dentata, 250
- Morisoni, 250
- Olitoria (corn salad), 250
- rimosa, 250
- Vinca minor (periwinkle), 13, 21
- Volvaria volvacea Bull., 57
- Volvex, 133
- Wakefield, E. M., see Dennis, R. W. G.
- Ware, W. M., see Glasscock, H. H.
- Webb, R. A., 'The microbiology of some African native beers', 95
- Williams, D. A. & Hyde, H. A., 'A daily census of atmospheric *Alternaria* spores at Cardiff during 1942 and part of 1943', 95
- Wilson, A. R., see Boyd, A. E. W.
- Wilson, I. M., 'Observations on wilt disease of flax', 221
- Wilson, M., 'Alpine rusts in Scotland', 189
- Xylaria Hypoxylon (L. J. Grev.), 57
- polymorpha (Pers. ex Fr.) Grev., 43
- Zygorhizidium Willei Loewenthal, 133
- Zygosporium parasiticum (Grove) Bunting & Mason, 97